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The Sustainable Remediation of Abandoned Lead Mine Tailings

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The University of Edinburgh

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Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree or professional qualification. Except where stated otherwise by reference or acknowledgment, the work presented is entirely my own.

Andrew Innes

April 2019

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Abstract

The extraction of lead (Pb) has occurred throughout Europe for over 3,000 years, bringing substantial benefits to economies, technological advances and the development of infrastructure. The relative ease of extraction and refinement of Pb, coupled with its usefulness in a range of applications resulted in extensive, and often unmanaged, exploitation of this element. The environmental legacy of historic Pb mining activities are evident throughout Europe, with hundreds of abandoned mine tailings documented throughout the EU and in the UK. These mining sites often present a risk of point and diffuse source pollution, with the main issues occurring from the introduction of potentially toxic elements (PTE) into the surrounding environment.

Abandoned (Pb) mines are often inherited by new landowners who are consequently liable for their management, generating a burden of responsibility that may involve restorative action. Unfortunately, the cost of conventional remedial strategies is frequently prohibitive for small landowners to undertake, leading to the possible continued dereliction of these sites. Identifying economically feasible and effective solutions to this issue is therefore of importance. Critically, reducing the risk of PTE impacts to the environment is pertinent, as are endeavours to convert these derelict mines into more productive landscapes. Therefore, the overarching aim of this thesis was to further knowledge on ecologically driven remedial approaches to metalliferous mine tailings to lessen the economic issues faced in environmental protection. In particular, the thesis aimed to ascertain if it was feasible to use native plant species and locally available waste materials to promote the revegetation of abandoned Pb mines, and consequently provide a sustainable vegetative barrier that could limit PTE release into the surrounding environment. The investigation was centred on two abandoned Pb mines, located on the West coast of Scotland (Tyndrum) and the South of France (Le Bleymard).

Geo-statistical investigations identified elevated concentrations of total Cd, Pb and Zn at both sites combined with a lack of organic matter, plant nutrients and

poor physical structure conditions for vegetation. Contrasting findings were identified, with the Tyndrum tailings exhibiting a higher potential mobility of PTE than Le Bleymard and a particular risk of hydrological movement of PTE enriched material from the mine to the wider environment. In opposition, the calcareous nature of the Le Bleymard tailings minimised PTE mobility via surface absorption and precipitation mechanisms, with the greatest risk to the surrounding environment likely caused by the aeolian transportation of fine particulates and inhalation of PTE enriched material. The use of inverse distance weighting and empirical Bayesian kriging highlighted the heterogeneous distribution of PTE enriched material at Tyndrum and the movement of small ($<100\text{ }\mu\text{m}$), Pb enriched particulates at Le Bleymard.

A combination of laboratory and field studies highlighted the use of common bent (*Agrostis capillaris*) as a useful phytostabilisation species, which was observed to be tolerant of up to $10,000\text{ mg kg}^{-1}$ Pb, $12,000\text{ mg kg}^{-1}$ Zn, and 180 mg kg^{-1} Cd in plant tissues. Furthermore, this species was generally effective in reducing the transference of PTE from the roots to aerial compartments of the plant, therefore minimising possible PTE transference to the food web. It was found that laboratory studies involving the spiking of PTE into synthetic growth media substantially underestimated plant uptake of Pb, Zn and Cd in the field and so should be used with caution when informing field trials.

The addition of organic amendments into the tailings produced contrary results depending on the source of the organic amendment and the underlying tailing characteristics. Broadly, the introduction of organic amendments into the slightly acidic tailings at Tyndrum can elicit the mobility of Pb by complexation with soluble Fe compounds and organic ligands, whilst concurrently promoting the immobilisation of Cd and Zn. Over a longer period, the separate incorporation of spent coffee grounds (SCG) and green waste compost (GWC) reduced the concentration of weakly associated Pb by complexation with stable organic matter and Fe/Mn oxy-hydroxides by 36.9 and 28.9 % respectively. Furthermore, improvements in plant available nitrogen (N), phosphorous (P), microbial activity and organic matter were observed in both field and laboratory

settings, with the inclusion of SCG observed to have the largest effect across all properties. Contrasting results were found in the calcareous tailings of Le Bleymard, where the potential mobilisation effects generated by organic amendments to PTE were mitigated by the elevated pH and occurrence of adsorption and precipitation events, leading to initial net gains of increased tailing fertility and minimal PTE mobilisation. However, a loss of plant available and total N coupled with the immobilisation of soluble P was observed, which was partially attributable for the stunted growth of *A.capillaris* during field trials when comparing the study sites.

The use of *A.capillaris* and the incorporation of organic amendments in the potential remediation of two contrasting abandoned Pb tailings were found to be substantially influenced by the underlying geo-chemical characteristics of the mine tailings. In conclusion it was suggested that the re-vegetation of the Tyndrum tailings could be achieved using SCG or a similar material which would provide a source of plant and microbial support combined with a high capacity for stable complexation of PTEs with C compounds. For Le Bleymard, suggesting a suitable material is more difficult due to conflicting needs to balance the risk of PTE release via the acidification of the tailings and enhancing the availability of plant nutrients. Overall, it was found that the use of *A.capillaris* combined with SCG or a comparable organic material could be highly effective in revegetation efforts at the Tyndrum site. However, wider investigations are suggested to be undertaken to identify a suitable material in the promotion of vegetation at the Le Bleymard tailings. It can be concluded that this work has furthered our understanding of ecologically driven rehabilitation of abandoned mine tailings and has highlighted the need for more work to be completed on calcareous mine tailings.

Lay Abstract

The usefulness of the chemical element lead has resulted in the widespread extraction of its ore, with the unintended consequences of substantial environmental impacts due to the mismanagement and abandonment of mining wastes. Combating the environmental impacts of abandoned mine wastes can be challenging due to the hazardous nature of the material and the cost involved in conventional decontamination processes. Currently, the scientific community is interested in ecologically mediated remedial approaches to return derelict mining landscapes to more useful systems.

This work selected two abandoned lead mines on the West coast of Scotland and the South of France as study sites. These were used to investigate the usefulness of specialist plant species and locally sourced organic amendments materials to alleviate the environmental impacts posed by the mines, and to establish if a protective vegetative layer could be grown on the mine wastes to minimise pollutant movement. It was found that the grass species *Agrostis capillaris* appeared to be a suitable candidate for the revegetation of the mine wastes and could tolerate the combination of high concentrations of toxic metals and low nutrient presence in these environments. It was observed that the effectiveness of organic amendments materials varied considerably depending on the underlying nature of the waste material and the chemical characteristics of the mine wastes. For the Scottish site, it is suggested that used coffee wastes or a similar material could be useful in the environmental improvement at this location, whereas the French site was more complicated due to conflicting issues of increasing plant establishment and the risk of exacerbating metal pollution to the surrounding environment.

Further work was suggested to be completed on mining sites similar to the French mine to understand the extent of these issues, whereas for sites similar to the Scottish mine a more generalised approach could be adopted, and larger field trials could be undertaken based on the findings of this work.

Abbreviations

ANOM	Analysis of means
ANOVA	Analysis of Variance
BC	Bioaccumulation coefficient
BCR	Community Bureau of Reference
CM	Cattle manure
CWEC	Cold water extractable carbon
DC	Direct current
DEFRA	Department for Environment, Food and Rural Affairs
DOC	Dissolved organic carbon
EBK	Empirical Bayesian kriging
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetic acid
EE	Extraction efficiency
FID	Flame ionisation detector
GWC	Green waste compost
HWEC	Hot water extractable carbon
ICP-MS	Inductively coupled plasma mass spectroscopy
ICP-OES	Inductively coupled plasma optical emission spectroscopy
IDW	Inverse distance weighting
LMWOA	Low molecular weight organic acid
LOD	Limit of detection
LOI	Loss on ignition
LOQ	Limit of quantitation
MANOVA	Multivariate analysis of variance
MBC	Microbial biomass carbon
NVC	National Vegetation Classification
OM	Organic matter
PCA	Principal component analysis
PS	Particle size
PTE	Potentially toxic element

RF	Radio frequency
SCG	Spent coffee grounds
SM	Sheep manure
SRUC	Scotland's Rural College
TC	Total carbon
TCD	Thermal conductivity detector
TF	Translocation factor
TN	Total nitrogen
WHC	Water holding capacity

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Table A 27. Pearson’s product-moment correlation coefficient for Zn measured parameters and tailing characteristics at the Le Bleymard tailings. WS-P = Water soluble phosphorous. Correlation co-efficients greater than 0.7 or less than -0.7 are highlighted in bold.	409

Chapter 1. Introduction and Aims

1.1 Introduction

Metalliferous mining activities have occurred for approximately 3,000 years across Europe, contributing enormously to economic progress and industrial development, particularly in the past 200 years. However environmental legislation relating to these activities has only realistically existed since the 1990 Environmental Protection Act (British Geological Survey 2017), creating a substantial legacy of point source and diffuse pollution from unregulated mining activities and the disposal of waste material enriched with potentially toxic elements (PTE) into watercourses, the atmosphere and potentially productive land (Milton *et al.* 2002; McGrath *et al.* 2004; Yang *et al.* 2008).

Considering metalliferous mining, the extraction of Pb was arguably the most prolific over the previous 300 years, owing to the high demand of Pb for several products, such as in water distribution systems, pigments, pottery, and more recently in batteries, petroleum and radiation protection (De Keersmaecker *et al.* 2018). As a result, excavation and ore extraction for Pb throughout Europe was significant and led to numerous mining operations ranging from small artisan mines such as in Le Bleymard (S. France), to large activities as seen in Leadhills (W. Scotland) (Rowan *et al.* 1995; Cloy *et al.* 2005; Pickin 2010). Due to the previous lack of environmental regulation the waste materials were often dumped in large unmanaged heaps (designated ‘tailings’) or discharged directly into watercourses to dispose of the waste directly. Focusing on the former, despite extractive endeavours, the waste material could still contain up to 6% w/w Pb. The processed tailings are often present as crushed, sandy material. This can exacerbate transportation into the wider environment, present an increased weathering potential and greater bioavailability of pollutants as compared to the original rock (Pulford *et al.* 2009; Hayes *et al.* 2012).

The abandonment of historic mining activities presents several problems; tailing wastes and their environmental management issues are usually inherited by new landowners, such as is the case of the Forestry Commission and the independent dairy farmer who own tailing heaps in Tyndrum (Scotland) and Le Bleymard

(France) respectively. In both situations the original mining companies no longer exist, and so retrospective compensation for remedial activities to the current landowners is no longer possible. Furthermore, the cost of remediation of Pb contaminated land can range from £15 - £1,500 m³ of soil depending on the treatment technology used (CL:AIRE 2010; FRTR 2014). This presents a significant financial burden on the proprietor to meet legislative requirements if the tailings pose a risk to the environment. This cost may then be exacerbated when tailing dumps are located in rural areas, due to greater transportation costs, lack of infrastructure and limited access to electricity and remedial resources.

Research into ecologically driven remedial processes have been conducted for over 25 years, with thousands of studies assessing the suitability of a range of plant species, organic amendments and novel remedial techniques to reduce environmental impacts and improve ecological conditions on contaminated soils and mine tailings. The incorporation of waste materials into Pb/Zn tailings presents an attractive solution for remediation. However, the chemical and physical properties of organic amendments vary substantially, and therefore could have a wide range of impacts to mine tailings, including immobilisation of PTE, increased leaching of plant nutrients, or enhanced bioavailability of PTE depending on the chemical characteristics of the waste material and the interactions with the tailings themselves (Park *et al.* 2011; Branzini and Zubillaga 2012). It is important therefore that remedial approaches consider the properties of the organic amendment and the possible interactions that may occur when introduced to diverse mine tailings.

Despite a significant depth and breadth of research in this subject, uptake by industry or actual realisation of remedial approaches remains low. This can be partly attributable to several factors, such as availability of resources, geographical location of mine tailings, and the complexity and diversity of underlying geochemical characteristics of abandoned tailings. For example, the use of highly effective manganese (Mn) substrates as Pb immobilisation agents (McCann *et al.* 2015) are only economically viable if the materials are available in sufficient quantities, have a low processing cost and are in close proximity to

the tailing areas to avoid excessive transportation. Furthermore, due to the high geochemical and environmental variability in and around tailing systems it is difficult to assess the generalisable effectiveness of organic amendments. For example, it is unclear if one particular source of organic amendments or materials with similar chemical characteristics are useful in the amelioration of potential pollution and improve tailing fertility across several tailing parameters, or if specific pairing needs to be made between organic amendments and mine tailings. Moreover, the use of native plant species that can tolerate low nutrient conditions combined with a resistance to PTE induced stress or death are essential in revegetation efforts. Although significant focus has centred around plant species that remove PTE from tailing systems via absorption by roots and subsequent harvesting of the plant biomass, a shortage of identified and tested species that can provide a phytostabilisation cover remains. A greater identification of these species and evidence of their usefulness in phytoremedial projects is key to support ecologically driven remediation and wider uptake in large scale projects.

Finally, investigations into the geochemical nature, impact to the environment and remediation of mine tailings often consider only one tailing material at a given time, reducing the information available on the underlying processes and controlling factors that may be apparent across multiple mine tailings at a particular site. Furthermore, the differences in investigative approaches, materials used, and analytical procedures vary widely between studies, reducing the comparability of findings and generalisation of ecologically driven remediation approaches to the wider scientific literature.

1.2 Aims

The overarching aim of this Ph.D. was to further knowledge on ecologically driven remedial approaches to metalliferous mine tailings. In particular, the Ph.D. aimed to ascertain if it was feasible to use native plant species and locally available organic amendments to promote the revegetation of abandoned Pb/Zn mines. The investigation was centred around two mine sites with similar socio-

economic impacts but contrasting environmental conditions and underlying geological characteristics.

1.3 Objectives

The specific objectives of the Ph.D. are as follows:

1. To understand the pollutant pathways and chemical characteristics of each tailing site and produce novel information on the perceived risks to the wider environment and inform future experiments relating to ecologically based remedial approaches.
2. To identify tolerant plant species within the local environment that could be suitable for the re-vegetation of the mine tailings.
3. To assess the effectiveness of locally available organic amendments in the reduction of PTE mobility and enhancement of tailing fertility and microbial functioning over an initially short-term period (28 days).
4. To assess the viability and transferability of laboratory investigations into field environments, appraising the usefulness of laboratory experiments in the prediction of field scale trials.
5. To compare and contrast the impacts of remedial approaches in two contrasting geochemical and environmental settings to aid the understanding of underlying processes, caveats and challenges in generalising remedial approaches.

1.4 Research Activities

The research activities and connection of experiments of the project are described in Figure 1-1 below.

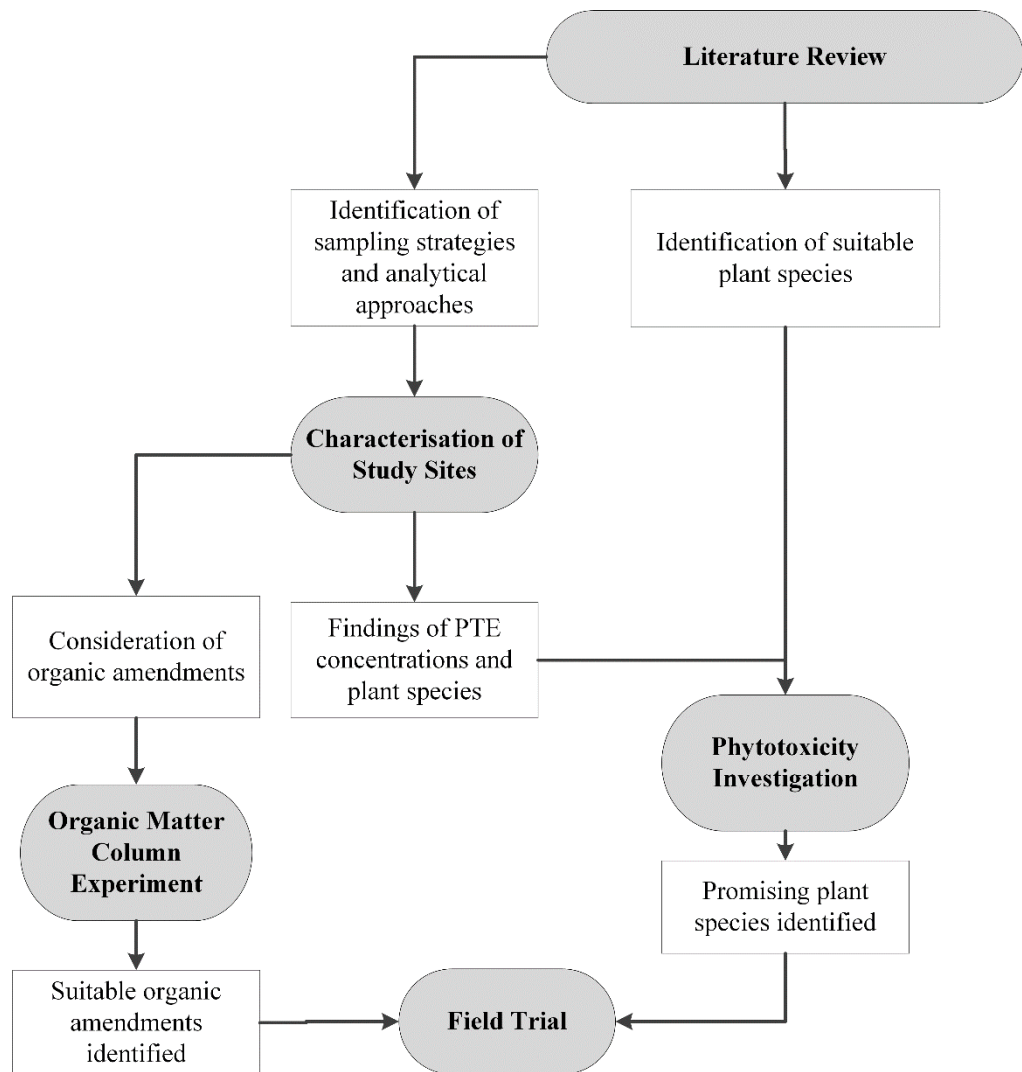


Figure 1-1. Conceptual diagram of research work covered in the thesis.

Chapter 2. Literature Review

2.1 Introduction

This chapter introduces the relevant subject areas that will be explored in subsequent chapters of this thesis. This chapter focuses on the background of historic mining processes and the legislative evolution that has occurred in the UK and France relating to the management of mining activities. Furthermore, this section will present a review on the current literature surrounding the tailing heaps at Tyndrum and Le Bleymard, and a specific overview of cadmium (Cd), Pb and Zn as PTEs of environmental risk due to their elevated presence in the study sites. Finally, a brief review on the current remedial approaches for abandoned mines is given, with particular reference to phytoremedial approaches, organic amendments and microbial sequestration.

2.2 Abandoned Mines: Legislation and Environmental Policy

The UK and France face similar issues, where historical mining activities have burdened current landowners through a lack of environmental management during the operational phases, and negligible remedial efforts upon mine closure. Difficulties arise in the management of these landscapes due to issues such as the transportation of pollutants to the wider environment if the tailing material is disturbed and the challenges faced in remediating the material. Furthermore, the landscapes generally remain unproductive and require consistent monitoring or management to protect both the public and environment, producing a financial burden on the proprietor.

2.2.1 UK Policy and Legislation

Historically in the UK mining legislation was only enforced or created after significant incidents or disasters, and mainly surrounded the working conditions of employees or extraction procedures (British Geological Survey 2017). Environmental legislation was not introduced until the passing of the Mineral Working Act in 1951, as such, land owners possessed full autonomy of the mining activities and the discharge of waste materials to soils, watercourses and the atmosphere. This has resulted in substantial contamination of the environment via dumping of waste materials such as tailing material onto soils,

the use of watercourses to remove flocculated materials and the emission of airborne particulates of smelting e.g. (Hudson-Edwards *et al.* 1997; Cloy *et al.* 2005; Rodríguez *et al.* 2009; Küttner *et al.* 2014; Mighall *et al.* 2014; Mills *et al.* 2014).

The Mineral Working Act (1951) enabled local authorities to reclaim a proportion of the capital gained from the mine to allow restorative works to be completed after activities ceased, although this act only specifically addressed the mining of ironstone. Restoration was generally aimed at recovering land for agricultural or commercial forestry purposes, rather than for ecological functioning or to promote environmental regeneration. Further statutes that indirectly challenged the environmental care of metalliferous mining came from the Town and Country Planning act (1962), where previously no planning permission was required for mining activities. The Act initiated further environmental considerations that the mining industry had to address, although this was mainly focused on aesthetic demands and landscaping to reduce landscape scarring.

The most substantial piece of legislation in UK history was generated from the Aberfan disaster in 1966, where 116 children and 28 adults died from poorly managed coal mine tailings. From this, the Mines and Quarries (Tips) Act 1969 was created, focusing on the stability of disused mine tips and requiring owners and responsible parties to ensure they did not pose a risk to public safety. This management requirement likely reduced other possible environmental impacts by reducing the movement of potentially harmful particulates into the wider environment.

Currently mining waste activities are overseen according to Directive 2006/21/EC of the European Parliament and the Council on the management of waste from extractive industries, (Council of the European Union 2006) with the legislation transposed to UK law. The 2006/21/EC Directive mandates that wastes resulting from extractive industries must not negatively impact soil, water or air quality as well as human health. Substantial waste management plans, environmental impact assessments and monitoring systems are required for

current extractive processes, significantly reducing environmental degradation from current mining activities. In the Directive, no specific licencing system exists for metalliferous mines, but planning permission and extensive environmental impact assessments are required for all proposals.

With specific reference to abandoned metalliferous mines in the UK, no national organisations are tasked with the management of these landscapes unlike the coal industries, where the Coal Authority have overall responsibility. Thus, no significant financial or policy support has been available, leaving local authorities to carry the costs of remediation (Johnston *et al.* 2008). Key areas and specific waterbodies such as the Dee, Humber and Severn have been identified by DEFRA with £10 million set aside to manage impacted sections of these priority water courses (CL:AIRE 2014).

2.2.2 French Policy and Legislation

French law states that ownership of land gives the proprietor the rights to minerals both below and above the ground in that area. Initially, mining rights and management of the activities were governed by local barons or regional lords until the Napoleon reform in 1810, where the control and governance of mining activities were transferred to the state. The *Code civil des Français* (1804) states a list of resources that are managed by the state (most minerals), and so require permission to be extracted.

Ownership of the geographical location was complex, with the original landowner retaining the rights to the area, but not necessarily to subsoil minerals, which would be allocated to a concessionary from the state to a chosen body. The owner may not automatically have priority when applying for the mining rights, although they may receive a small licence fee if they were not successful in an application (*Code civil des Français*, 1804). This legislative act technically created a new property, which could be independently sold, although control and final authority remained with the state (*Code civil des Français*, 1804). Currently, the same broad legislation applies although improvements have been made in terms of compensation for landowners and management of the post mining landscape (Clement *et al.* 2017).

Environmental stewardship in relation to metalliferous mining in France was essentially non-existent with pollution control only considered in relation to immediate human health impacts. Concerns of environmental damage from mining gained traction slightly later here than in the UK, when France underwent substantial economic and industrial changes in the early 1990's. The first major pieces of legislation occurred from the Water Law (1992) and Air Law (1996) which were further strengthened under the Environmental Charter (2004). The charter establishes a blanket policy for environmental awareness, risk assessment and impact abatement across all activities. Recently the Mining Code was updated in January 2017 to encompass values from Directive 2006/21/EC and the Environment Charter (2004) to promote a more sustainable and environmentally conscious mining industry.

Prior to the environmental reforms, upon the conclusion of mining activities ownership of the land was returned and the operator was charged with managing 'annoyance' from the activities, usually by controlling discharge of waste materials to nearby waterbodies. As of 2004 a risk-based approach was adopted, with mining operations requiring a suitable closure plan and the capital to achieve this. This risk is to be evaluated by the State and must consider regional planning. If the operator has gone into administration or is no longer a viable business then the State is to assume responsibility (Environment Charter 2004).

Despite legislation conferring final ownership and remediation charges to the state, a legacy of abandoned mines continues to pose a pollution issue across France e.g. (Roussel *et al.* 2000; Casiot *et al.* 2007, 2011; Resongles *et al.* 2014). Several reasons may exist for this, including inaction where acute pollution risks are considered low, lack of financial backing and public support of mining heritage. Similarly to the UK, several abandoned mines are privately owned with the landlords obliged to manage the landscapes and manage the existing hazards left by the operators following the cessation of mining processes such as extraction and/or smelting.

The usefulness of Pb both historically and in present day society has led to pervasive ore extractions and mining activities for this element (Alloway 2012).

Therefore, the policy instruments explored in this section will have had a direct bearing on the emission of Pb to the wider environment from mining processes. Considering the weak legislation surrounding mining activities in general until recent years, unregulated Pb extraction processes have had a substantial global impact on environmental and human health (Dudka and Adriano 1997; Liu *et al.* 2005; Zhang *et al.* 2012; Li, Ma, *et al.* 2014).

2.3 History of Abandoned Pb Mines

2.3.1 Ore Extraction, Crushing, Floatation and Separation

The extraction and smelting of Pb has occurred for thousands of years, with several independent civilisations including France and the UK utilising the straightforward extraction processes of crushing, sedimentation and heating to obtain Pb for a range of purposes (Thornton *et al.* 2001). Extraction procedures moved from simple kilns to larger operations around the 1700's when industrial methodologies were developed (Figure 2-1) and there was a greater demand for Pb to supply the plumbing, glazing and painting industries (Thornton *et al.* 2001). Open cast mining for Pb was preferred, where drilling and blasting large areas of rock enabled the extraction of the target ores, mainly galena (PbS) and occasionally pyromorphite ($\text{Pb}_5(\text{PO}_4)_3\text{Cl}$). Several supportive landscaping activities were required to facilitate the mining, such as slope excavation, creating substantial disturbance of the environment. (Rich 1994). Post mining processes for the extraction of Pb necessitate a particle size <0.15 mm for the purposes of separation and concentration of Pb rich material, thus substantial milling and crushing of ores was required. The subsequent separation stage involved a complex series of flocculation, floatation and depression of the milled material using several chemical reagents that targeted specific mineral grain sizes and densities, selecting for material that contained approximately 55% Pb w/w (Rich 1994; BCS 2002).

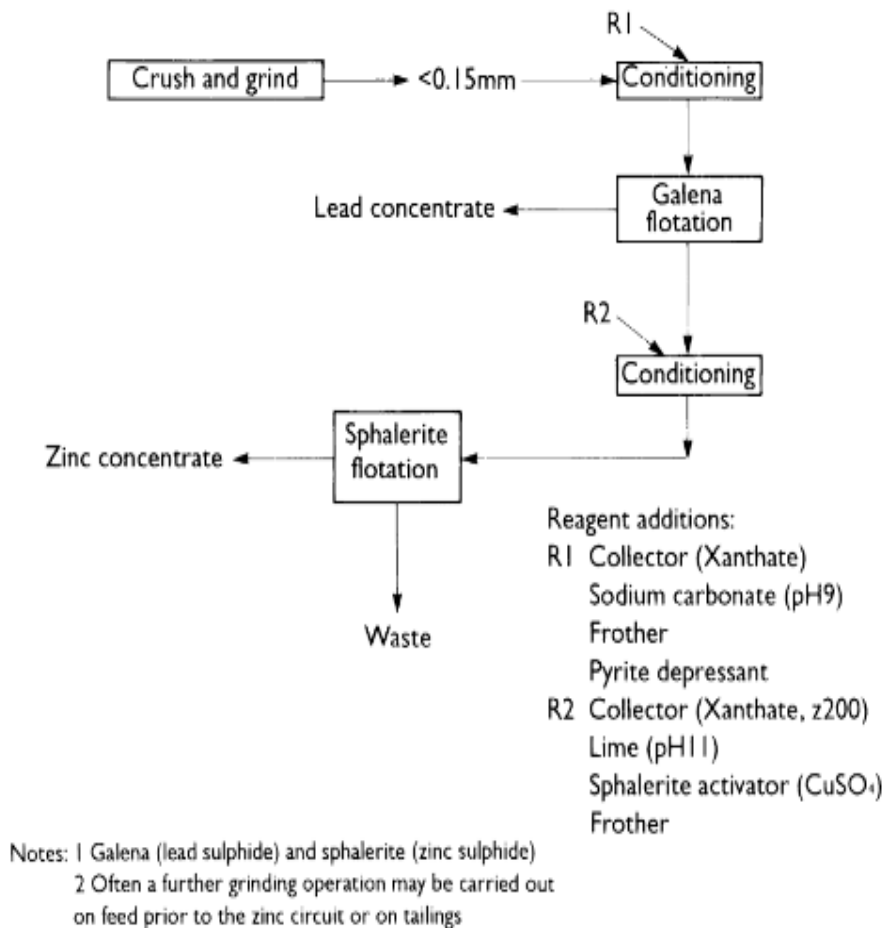


Figure 2-1. Overview of Pb ore processing systems. Adapted from (Rich 1994).

The waste material from the separation processes were usually dumped in large, unlined tailing piles (Figure 2-2) and occasionally reprocessed when newer technologies made further reprocessing for Pb economically viable. Despite reprocessing efforts the waste material was often still enriched with Pb, with contents of up to 6 % w/w in some instances (BCS 2002; Mills *et al.* 2014).

To date similar extraction and processing methodologies are still applied, but often with greater efficiency and substantial reworking of the tailing material to recover as much metal as possible. Furthermore, the excavated material is often processed for several other elements, such as Zn, silver (Ag), and copper (Cu), reducing the concentrations of PTE in the resulting waste material and making the overall extraction process more economically feasible (Rich 1994).



Figure 2-2. Tailing piles at the abandoned Pb/Zn mine of Le Bleymard.

2.4 Human Impacts and Environmental Issues

Abandoned mines have had a significant impact on human and environmental health, mainly through the transportation of PTE enriched particulates from bare tailings into soils, watercourses and the atmosphere e.g. (Concas *et al.* 2006; Corriveau *et al.* 2011; García-Giménez and Jiménez-Ballesta 2017). Over the past 40 years hundreds of studies have assessed the impacts to humans and the environment, documenting cases of direct Pb poisoning from tailing materials in humans (Yabe *et al.* 2015; Mills and Adderley 2017) and livestock (Sharpe and Livesey 2006; Rodriguez-Estival *et al.* 2012; Pareja-Carrera *et al.* 2014), pollution of agricultural lands (Li *et al.* 2006; Williams *et al.* 2009; Boussen *et al.* 2013) and impacts to a range of wildlife (Reglero, Taggart, Castellanos, *et al.* 2009; Reglero, Taggart, Monsalve-González, *et al.* 2009; Beyer *et al.* 2013; Duarte *et al.* 2014).

The Pb/Zn mines at Le Bleymard and Tyndrum have been documented to have a legacy of pollution episodes, impacting the surrounding environment and the health of mine operators and local communities.

2.4.1 The Tyndrum Pb/Zn Mine: Human and Environmental Impacts.

The Pb/Zn mine tailings at Tyndrum (Figure 2-3) were originally opened upon the discovery of Pb ore by Sir Robert Clifton around 1740 and worked extensively by the Scots Mining Company between 1768 and 1791 (Mills *et al.* 2014). From this period onwards, mining activities were sporadic, with several changes in ownership and extraction approaches until the final closure of the mine in 1928 after minor re-processing efforts by the Tyndrum Pb and Zn Company (Mills *et al.* 2014). Abandonment of the mine has continued since then under the current ownership of the Forestry Commission (FC) and joint management under the FC and Historic Environment Scotland. Currently, the 1.1 ha site is predominantly used for scientific investigations and informal recreation, such as walking and motocross sports.

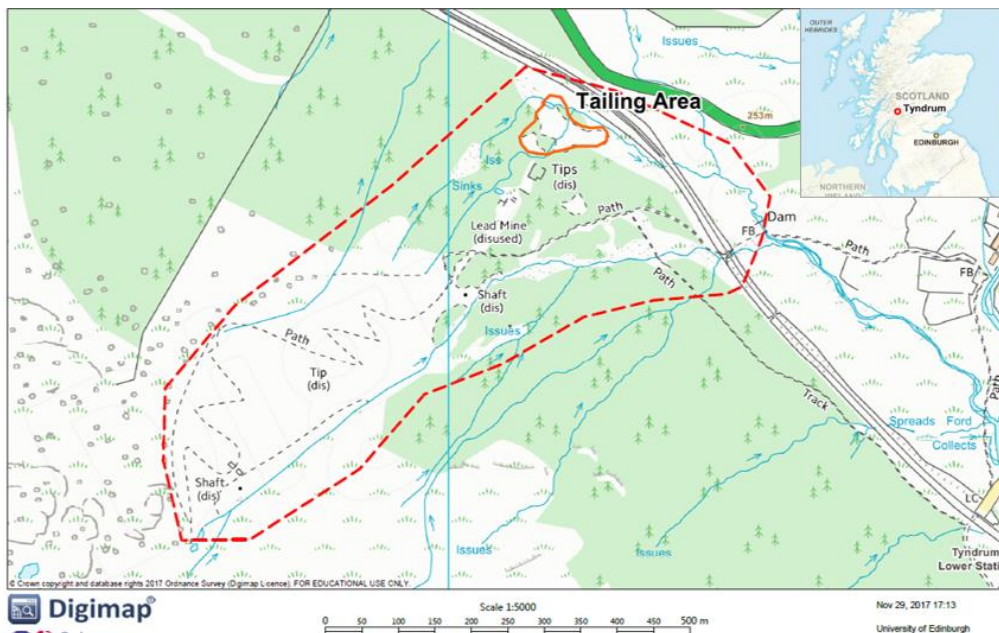


Figure 2-3. Location of the Tyndrum Pb/Zn tailings in Western Scotland.

The mine tailings and surrounding environment have been subject to several scientific studies, most notably by Farmer *et al.*, (1997) who linked Pb concentrations in Loch Tay to mining and reprocessing activities at Tyndrum, located approximately 25 km upstream. Further investigations have mainly focused on the transportation routes of Pb and Zn from the mine, such as aeolian dispersal mechanisms (Mansor 2006) and hydrological means (MacKenzie and

Pulford 2002; Pulford *et al.* 2009). From these studies it is recognised that PTE pollution from the mine is predominately attributable to the movement of the mine tailing material via surface runoff and sediment transport to waterbodies, with aeolian dispersion of PTE enriched particulates not posing a significant pathway risk. Moreover, concentrations of Pb and Zn in the dissolved fraction ($<1.2\ \mu\text{m}$) of the surrounding tributaries have been reported to be within the range of 0.3 – 2.5 and 0.5 - 4.0 mg L⁻¹ respectively (Mansor 2008), further suggesting that pollution from the tailings are mediated via particulate movement rather than in a dissolved form.

Chemical assays undertaken on the streambed sediments from the tailing area and further downstream have identified that Pb is predominantly held in the weakly associated fraction as described by the BCR 2001 protocol (Pulford *et al.* 2009) which raise concerns surrounding potential release of Pb into the environment, specifically if the mine tailing material is exposed to changes in redox or environments with a pH <5.0 . This high potential availability is attributed to the weathering of unconsolidated galena and anglesite, leading to the formation of Pb oxide carbonate materials (e.g. $\text{PbCO}_3\text{-PbO}$ and $\text{PbCO}_3\text{-2PbO}$). Additionally, it has been noted that the risk of Zn release from the mine tailing material is less likely due to the majority of the element reported to reside in the reducible and oxidisable fractions (as defined in the BCR protocol, 2001) of the tailing matrix and thus, considerable weathering or aggressive chemical change would be required to release these pools of Zn for biotic interaction (Pulford *et al.* 2009).

A recent study from Mills and Adderley, (2017) explored the possibility of incidences of negative health impacts resulting from the Tyndrum mining activities. The investigation highlighted that Pb poisoning would have been expected to be prevalent at Tyndrum, with evidence for a juvenile workforce, a greater incidence of air pollution due to less efficient smelting processes and a greater bioavailability of Pb in the tailing material (Figure 2-4). One documented case was shown in 1784 where mining activities were halted due to a poisoning incident during a period of intensive Pb production (Mills and Adderley 2017).

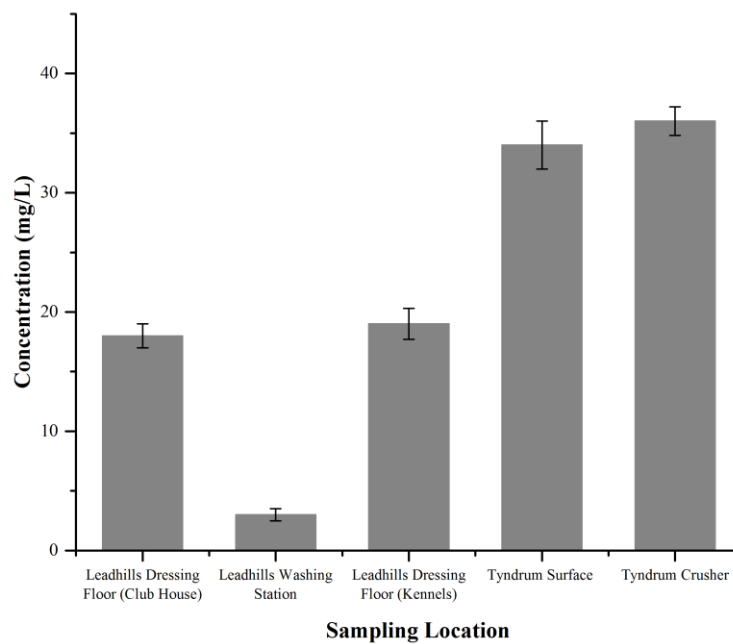


Figure 2-4. Concentrations of synthetic gastric acid-extractable Pb from samples originating from Leadhills and Tyndrum. Adapted from (Mills and Adderley 2017).

However, Mills and Adderley, (2017) did not find substantial evidence of Pb related health issues in Tyndrum which they primarily attributed to irregular mining operations caused by poor weather, a workforce committed simultaneously to agriculture as well as mining and lower outputs of Pb in comparison to the activities at Leadhills. The authors concluded that the population were likely to have suffered from Pb poisoning, however the symptoms may not have been diagnosed due to a lack of medical facilities and the absence of continuous, high exposure to Pb enriched particulates compared to Leadhills.

Overall, the mine tailings at Tyndrum have been identified as a key source of Pb into downstream waterbodies, with some historic impact in terms of Pb enriched particulate inhalation and health issues in the village of Tyndrum. Arguably, the largest concerns of Pb release to the surrounding environment are focused around the surface erosion of the tailings and the potential changes in environmental conditions which could elicit substantial release of Pb into aquatic

systems. To date, only one minor attempt at revegetation of the site by the FC has been attempted, where several native species of trees were established on the tailings (Mills *et al.* 2014). The project was largely unsuccessful as most of the trees died and did not appear to improve the tailing nutrient content or promote the wider revegetation of the tailing heaps.

2.4.2 The Le Bleymard Pb/Zn Mine: Human and Environmental Impacts

The Cévenne National Park in France has been influenced by mining activities since 300 BC, with valuable metals such as Ag and Pb extracted and processed throughout the region (Domergue and Leroy 2000; Baron *et al.* 2005; Domergue *et al.* 2006). Mining activities have also left a legacy of environmental pollution that has impacted both local and regional environments. Tailing runoff from abandoned mines has contributed to the enrichment of sediments in the Gardon watershed (2014 km²) with PTEs such as Pb, Zn, arsenic (As), thallium (Tl) and Cd (Resongles *et al.* 2014). This transportation of PTEs has been noted to impact wildlife, particularly wild brown trout (*Salmo trutta fario*) where developmental irregularities have been linked to elevated Cd and Pb in the liver (Monna *et al.* 2011).

The Pb mining site at Le Bleymard (Figure 2-5) (3.6 ha) has seen development since Roman times; however records indicate that it was first opened for commercial mining in 1903 (Ploquin 2010). Approximately 6,000 tonnes of ore were extracted annually throughout sporadic operations until closure and abandonment around 1963 (Bouladon 1960, 1977; Disnar 1996). During this working period extensive aeolian Pb pollution occurred, reducing the life expectancy of residents by 20 years (Ploquin 2010). Since official mining activities have ceased the mine was initially reworked to provide aggregates for road surfacing. This reworking was criticised for producing elevated Pb concentrations in the blood of local children and potentially affecting their future health (Ploquin 2010). Currently the mine is managed by a local dairy farmer who uses the land as a storage area for silage and agricultural equipment.

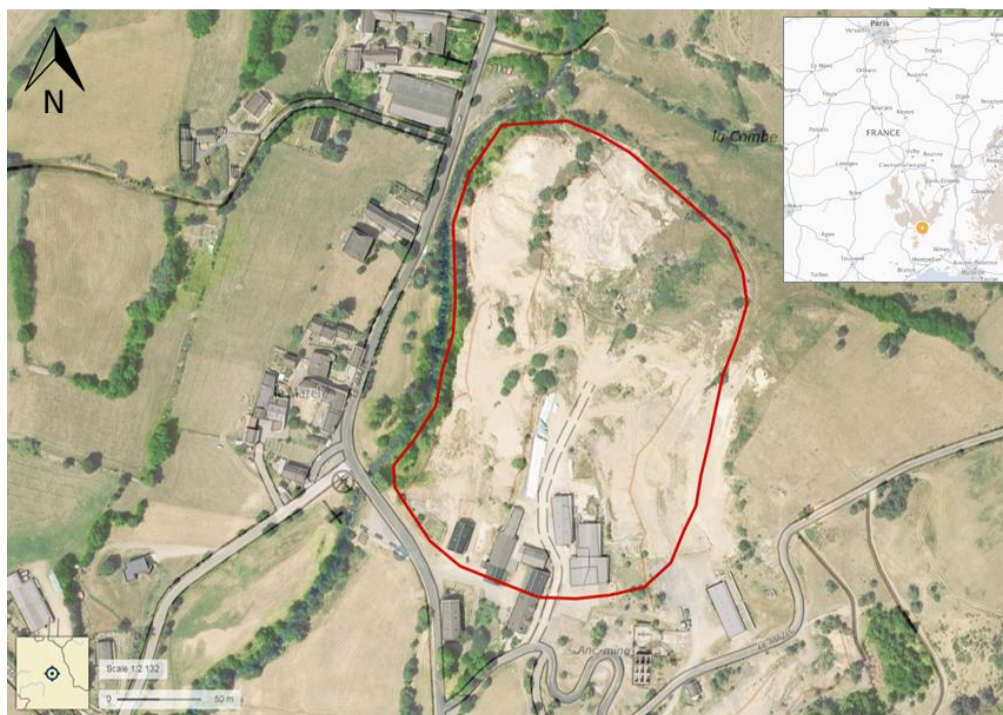


Figure 2-5. Location of the Pb/Zn mine at the Le Bleymard, South France. The tailing area is outlined in red.

There is a lack of scientific studies on the mine at Le Bleymard, with the most comprehensive available information describing mine tailing and root/shoot concentrations of the hyperaccumulator species Alpine pennycress (*Noccaea caerulescens*) for Cd, nickel (Ni), Pb and Zn, in each matrix, with tailing concentrations of 8.9, 0.01, 0.04 and 16.7% respectively (Reeves *et al.* 2001; Peer *et al.* 2003; Schwartz *et al.* 2006). Aside from earlier work regarding *T. caerulescens* (Wilkins and Williams 1977), other studies have only mentioned the mine in passing (Baron *et al.* 2005, 2006; Monna *et al.* 2011) with some reference to its historical activities or the potential for contributions to atmospheric Pb and discharges of Cd to the wider environment.

The stream running to the east of the tailings is occasionally used by educational groups to demonstrate the impacts of metal runoff on invertebrate diversity. Biodiversity reductions from the tailing runoff are assumed to be highly localised (Hillcox. S 2015), however no rigorous investigations have taken place to fully qualify this position. No remedial actions have occurred since

abandonment and so the mine continues to pose a potential environmental hazard.

2.5 Chemical Behaviours of Key Elements Investigated in this Study

2.5.1 Lead (Pb)

Chemical Properties and Occurrence

Lead is a naturally occurring, dense metal allocated to group 14 in the periodic table with an atomic number of 82 and an atomic mass of 207.1. Lead is most commonly found in the (II) oxidation phase, although under specific conditions, such as exceptionally low pH (<3), oxidation states of (IV) are possible. In general, Pb has low reactivity and forms covalent bonds when interacting with other elements, usually leading to the formation of stable compounds (Emsley 2011).

Lead has a global average abundance of 14.8 mg kg^{-1} (Hans Wedepohl 1995), with the majority of Pb associated with sulphur bearing minerals, such as black shales, organic rich substrates (Meyers *et al.* 1992) and to a lesser extent in silicate and carbonate minerals due to the substitution of Ca^{2+} and K^{+} by Pb^{2+} (Alloway 2012). The most commonly encountered ore of Pb is galena (PbS), however accumulations of pyromorphite ($\text{Pb}_5(\text{PO}_4)_3\text{Cl}$), and galena's oxidation and decomposition minerals anglesite (PbSO_4) and cerussite (PbCO_3) can be locally relevant in an increased Pb presence in the lithosphere (Hans Wedepohl 1995). Substantial exploitation of Pb has led to worldwide anthropogenic contamination of soils, primarily due to atmospheric deposition from human activities, such as smelting, coal burning and emissions from leaded petrol, leading to a mean worldwide concentration of 25 mg kg^{-1} in top soils (Emsley 2011). In particular, Pb is strongly associated with smaller particulate fractions when present in combustion processes, therefore facilitating the global spread of this element via industrial emissions (Gladney *et al.* 1976).

Toxicological Impacts

Lead is a xenobiotic element that has no currently understood role in biological functioning and produces detrimental impacts to human health. Several exposure routes for Pb exist (Figure 2-6), with the most common occurrences of Pb

poisoning resulting from the ingestion or inhalation of Pb enriched particulates. This leads to greater occurrences of Pb poisoning incidences in children due to playing in areas of contaminated soils, pica behaviour (the consumption of non-food items) and from Pb based paints on toys manufactured before the 1960s (Mushak 2011; Mateus-García and Ramos-Bonilla 2014; Huber and Ekvall 2017).

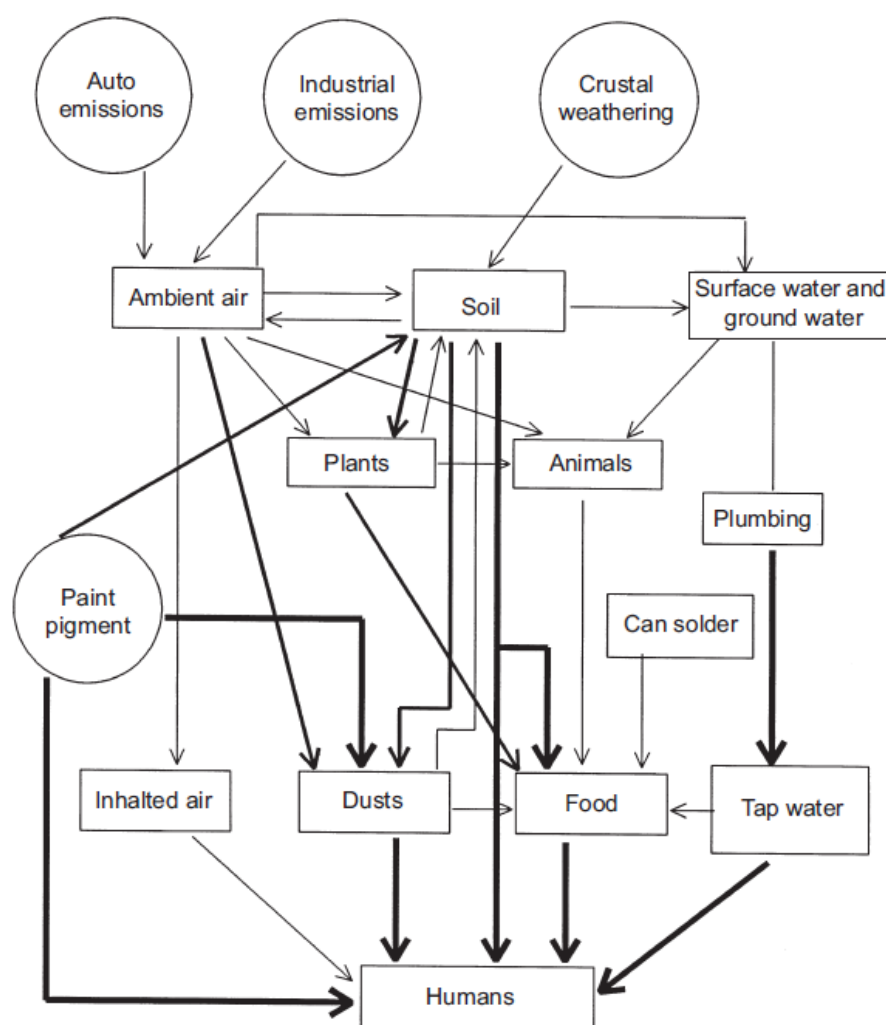


Figure 2-6. Pb exposure routes to humans, bold lines indicate risk pathways for children.

Adapted from Mushak, (2011)

Once Pb has entered the body it is distributed throughout the biological system, concentrating mainly in the skeleton (Barry 1975, 1981), but is also found in high concentrations in the liver, skeletal muscle, skin and brain tissues (Schroeder and Tipton 1968; Barry 1975, 1981; Gerhardsson *et al.* 1995). The

effects of Pb poisoning are well documented and include several acute and chronic illnesses, such as neurotoxicity in infants, kidney failure, anaemia and immunological suppression (Mushak 2011).

Behaviour in the Soil Environment

As previously mentioned Pb is most commonly found in the soil environment in the (II) oxidation state and is generally considered to be insoluble under aerobic conditions above pH 4 (McBride 1989). Furthermore, Pb has been shown to demonstrate a high affinity for inorganic and organic soil constituents such as clays, oxide minerals, carbonates, and in particular, organic matter (Alloway 2012). Lead has been widely shown to have a strong competitive effect for organic matter, with binding occurring across a range processes (Figure 2-7), predominantly forming inner sphere complexes that are typically stronger than other potentially toxic elements such as Cd, Zn, chromium (Cr) and Ni (Christl *et al.* 2001; Xiong *et al.* 2013).

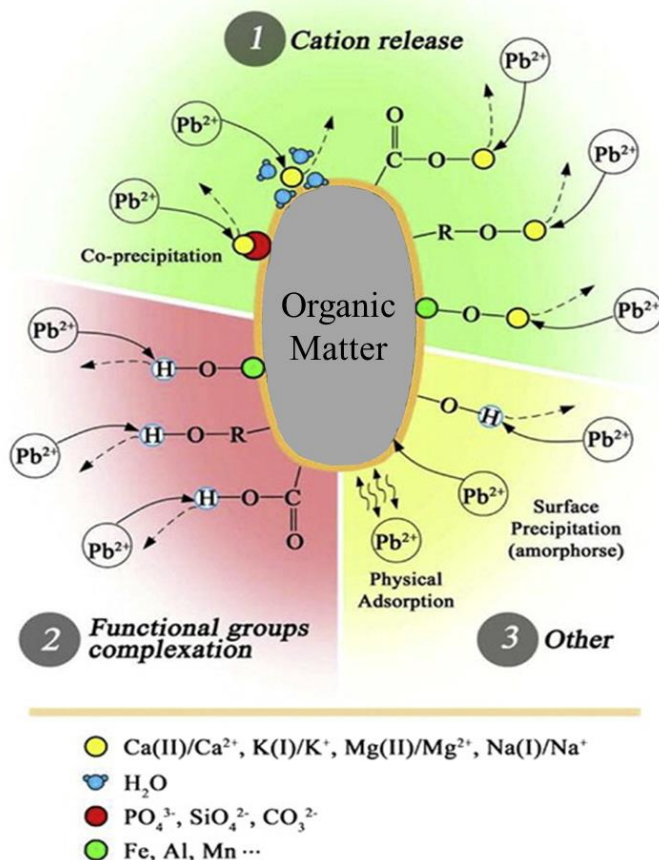


Figure 2-7. Potential mechanisms of Pb binding to organic matter. Adapted from Lu et al. (2012)

This high affinity for organic matter produces a strong immobilisation effect in most soils with appreciable concentrations of humic and fulvic material, leading to Pb characteristically residing in the regions of initial deposition, with limited transportation through the soil system (Li and Shuman 1996; Steinnes and Friedland 2006; Kobler *et al.* 2010). Conversely, in soil systems with an elevated pH (>8) or significant presence of dissolved organic matter, Pb can be mobilised due to the creation of soluble organic compounds and hydroxyl species (Harter 1979; Sauvé *et al.* 1998). In general the adsorption of Pb to organic matter results in a long term immobilisation of the metal, in the exception of microbial consumption and degradation of organic components and the formation of more soluble Pb species (He *et al.* 2015). Lead is also scavenged by aluminium (Al), iron (Fe) and manganese (Mn) oxyhydroxides and soil components with a high

negative surface charge, promoting immobilisation and a reduction in bioavailability for soil organisms (Hildebrand and Blum 1974; Kinniburgh *et al.* 1976; McKenzie 1980). These reactions tend to be stable over time unless redox and pH conditions fluctuate (e.g. flooding events, seasonal waterlogging, microbial influences and soil fertilisation) which can prompt the mobilisation of Pb into the soil solution (Calmano *et al.* 1993; Charlatchka and Cambier 2000a).

Owing to the immobile nature of Pb in most soil environments, the availability of this element to soil organisms and plants tends to be lower than some of the more mobile metal(loid)s such as As and Cd (Alloway 2012). Toxicity in microbial populations and higher soil organisms (e.g. earthworms) tends to be mediated by the ingestion or metabolic uptake of Pb compounds and the subsequent bioaccumulation within food chains, leading to higher Pb burdens in top-tier organisms (Reinecke *et al.* 2000; Nakayama *et al.* 2011; Rodríguez Martín *et al.* 2014). Plant uptake of Pb is often dependent on the soil characteristics and the species in question, however it is generally low and primarily facilitated via the release of plant exudates and low molecular weight organic acids which act as chelatants and promote Pb solubility (Shahid *et al.* 2012). The translocation of Pb to aerial, potentially edible plant compartments tends to be low due to the sequestration of Pb in the cell walls of the root system (Krzesłowska 2011; Xin *et al.* 2018). In a review of Pb transportation in plant systems Kumar and Prasad, (2018) suggested that plant systems generally counter oxidative damage from Pb to photosensitive pigment formation, DNA damage and protein oxidation via immobilisation reactions. This is mainly achieved via the production of antioxidants and lignins that reduce the activity of Pb radicals and promote the sequestration of Pb in the roots, where less sensitive plant biochemical reactions occur in comparison to the leaves and shoots.

2.5.2 Zinc (Zn)

Chemical Properties and Occurrence

Zinc is a biologically essential transition element with an atomic number of 30 and an atomic mass of 65.39. Zinc a known reducing agent, is almost exclusively

observed in the Zn^{2+} oxidation state and is chemically active, leading Zn^{2+} compounds to be important central ions in many complexes and biological organo-metallic compounds (Emsley 2011).

Zinc is a common soil element, with a global abundance in the earth's crust of 75 mg kg^{-1} and a range of $5\text{-}700 \text{ mg kg}^{-1}$ in soils depending on the underlying geology (Hans Wedepohl 1995). Notable Zn ores include sphalerite ($(\text{Zn}, \text{Fe})\text{S}$), smithsonite (ZnCO_3) and hemimorphite ($\text{Zn}_4\text{Si}_2\text{O}_7(\text{OH})_2 \cdot \text{H}_2\text{O}$), with the weathering of Zn containing ores contributing to a slow release into the soil matrix. Furthermore, the texture of the soil has an important bearing on Zn presence, with soils exhibiting high clay contents containing greater concentrations of Zn than sandy ones due to Zn adsorption onto these negatively charged surfaces (Baize 1997; Rinaudo *et al.* 2009). However, global anthropogenic enrichment of Zn in soils is a significant factor in elevated Zn concentrations due to high Zn demand from the galvanising, brass and electrical components industries (U.S Geological Survey 2017). Major routes of soil exposure originate from atmospheric deposition from mining and smelting, the leaching of Zn from galvanised infrastructure, application of sludge fertilisers (e.g. sewage sludges) and industrial waste water discharges (Gouman 2004; Zhang *et al.* 2011; Tlustoš *et al.* 2016). Despite the importance of Zn for biological processes, plant uptake of Zn generally does not exceed Zn additions to soils, leading to a net increase of total soil Zn over time (Keller *et al.* 1976; Moolenaar and Lexmond 1998).

Toxicological Impacts

Zinc poisoning in humans resulting from ingestion of food exposed to high soil concentrations of Zn are almost non-existent due to high toxicological thresholds (Oral $\text{LD}_{50} = 3 \text{ g kg}^{-1} \text{ bw}^{-1} \text{ day}^{-1}$) and the unlikely ability of dietary plants to supply such high concentrations (ATSDR 2005; Plum *et al.* 2010). Therefore, concerns surrounding elevated Zn in soils is focused on soil organisms and plants. When absorbed in plants Zn can produce chlorosis of leaves, inhibition of root development, and cellular oxidative damage (Ren *et al.* 1993; Fontes and Cox 1995; Chang *et al.* 2005), leading to a general reduction in plant

development. Several species of plants have countered these deleterious effects by sequestering Zn in non-essential biological compartments and removing them from important cellular regions of the plant (Rascio and Navari-Izzo 2011). In soil biota Zn poisoning has been documented to inhibit enzyme functioning in earthworms, functional diversity and enzyme production in microbial populations (Salminen *et al.* 2001; Lock and Janssen 2005; Zhou *et al.* 2009; Li *et al.* 2011), demonstrating direct toxicity when labile Zn concentrations are present in soil environments. In contrast, Zn deficiency in soils is a major agricultural problem in regions with highly calcareous soils, waterlogging or elevated phosphorous (P) and magnesium (Mg), which induces a decrease in Zn phytoavailability and a deficiency of the essential element in the food chain (Lindsay 1972; Orabi *et al.* 1981; Uygur and Rimmer 2000; Singh *et al.* 2005; Board 2008).

Behaviour in the Soil Environment

The chemical behaviour of Zn in the soil has been extensively studied, with a consensus that Zn is controlled by adsorption, complexation in the soil solution and phase changes between Zn compounds. Zinc is considered more labile than other trace elements found in soils (e.g. Cu, Pb, mercury (Hg)) (Alloway 2012), however, inner and outer sphere binding with humic compounds occur alongside weaker associations with clay sorption sites and Mn/Fe (oxy) - hydroxides (Roberts *et al.* 2002; Jacquat *et al.* 2009; Fan *et al.* 2016; Refaey *et al.* 2017).

An important phenomena when considering Zn behaviour in soils is the ‘ageing effect’, where over time, Zn bioavailability generally decreases as the element shifts into less available fractions, suggested to be as a result of diffusion of Zn^{2+} into oxy-hydroxide matrices (Gusiatin and Kulikowska 2016). This aging and immobilisation effect has been observed to increase directly with increasing pH (Brennan 1990) and is suggested to be a major controlling factor when considering Zn bioavailability in soils. Furthermore, Zn mobility is strongly associated with redox conditions, where Fe/Mn oxy-hydroxide bound Zn can be released if the dissolution of mineral components occur under waterlogged soil conditions. This leads to a direct release of Zn into the soil solution, but re-

adsorption may not follow as competition for liberated Fe (III) and Mn (IV) adsorption sites by other metals (e.g. Cu and Pb and Mercury (Hg)) may displace Zn into the soil solution (Degryse *et al.* 2004). Moreover, there is strong evidence that Zn solubility is highly dependent on the source and chemical form of Zn in the soil, with Zn originating from soluble salts, such as those from galvanised infrastructure promote greater mobility than those from geological origins (Degryse *et al.* 2004). Generally, it has been shown that soil physical and chemical properties are the dominating factors of Zn activity in soil solution, with soil texture and organic matter reporting to jointly account for less than 15% of the variability of the partitioning of this element (Azouzi *et al.* 2015; Rutkowska *et al.* 2015).

Overall, Zn is arguably less of a toxicological concern than other trace elements such as As, Cd and Pb, attributable to low comparative toxicity as compared to these elements and the uncommon instance of Zn poisoning via consumption of Zn enriched plants. However, elevated concentrations of Zn can have detrimental impacts on plants and soil biota and so contamination events should be managed for ecological protection.

2.5.3 Cadmium (Cd)

Chemical Properties and Occurrence

Cadmium is a transitional element with an atomic number of 48 and an atomic mass of 112.4. Cadmium is most commonly found in the Cd^{2+} state and forms a diverse range of inorganic and organo-metallic compounds (Emsley 2011), many of which are useful in the pigment, electroplating and battery industries.

Natural occurrence of Cd in soils is low, with a mean abundance of 0.1 mg kg^{-1} in the Earth's crust and a range of $0.1 - 1 \text{ mg kg}^{-1}$ found in global topsoil, subject to geology and human activity (Hans Wedepohl 1995). Naturally occurring Cd minerals are uncommon and mainly restrained to greenockite (CdS) and otavite (CdCO_3) (Llewellyn 1994), however these ores are rarely mined independently and so Cd is almost exclusively extracted as a by-product from Zn refinement (USGS 2017). Therefore, non-anthropogenic Cd presence in soils tends to be associated with black shales, sedimentary deposits rich in P and S and in soils

with high clay content (Alloway 2012). Due the low natural presence of Cd, enrichment of soils are heavily influenced by human activities, primarily via the use of phosphate fertilisers, sewage sludge applications and the atmospheric and fluvial deposition from mining and industrial activities (Andersson 1992; Bergkvist *et al.* 2003; Pacyna *et al.* 2009; Belon *et al.* 2012; Roberts 2014). This deposition of Cd has led to a consideration to limit Cd enriched fertilisers in 2002 in the EU (CSTEE 2002). Despite the limited uptake of this concept, the use of phosphate fertilisers in the EU decreased by 40% between 2000 – 2015, resulting in a direct reduction of Cd enrichment of EU agricultural soils (Six and Smolders 2014).

Toxicological Impacts

Cadmium has no currently known biological role in almost all organisms, with the element promoting serious health implications in human kidney function, reproductive ability and skeletal integrity (Godt *et al.* 2006). However, the ecotoxicological impacts of Cd are substantially less reported as compared to human issues, primarily because Cd soil concentrations of human concern are significantly lower than that which have a direct impact on plant and microbial communities (Alloway 2012). Difficulties in quantifying the impact of Cd to soil organisms arise from the lack of long-term field studies and the complex behavioural influences soil physical and chemical parameters have on Cd toxicity (Vig 2003; van Gestel and Koolhaas 2004). Furthermore, it is observed that crop biomass is not often severely impacted by Cd concentrations that are a risk to human health (He and Singh 1994; Ingwersen and Streck 2005; Sekara *et al.* 2005; Lamb *et al.* 2016), supporting the concept of Cd as mainly a human food-chain contamination issue rather than an ecological concern.

Behaviour in the Soil Environment

Vegetation can have a substantial effect on Cd behaviour due to the chemical similarity of Cd to Zn and Ca. Generally, uptake decreases with increasing pH, resulting from greater sorption onto soil surfaces and the creation of less mobile Cd-OH species (Lamb *et al.* 2016). Furthermore, ion competition effects and alterations of the rhizosphere by the introduction of plant exudates can have a

substantial effect of Cd uptake and mobility, mainly through complexation and chelation of Cd and a resulting increase in mobility (Sterckeman *et al.* 2005; Li, Liang, *et al.* 2013).

The electron configuration of Cd and the designation as a group B metal in the Schwarzenbach classification system generates a highly polarisable element, promoting greater mobility than most transition elements but stronger bonds than those of Ca^{2+} and Mg^{2+} (Petrucci 1971; Alloway 2012). Cadmium bioavailability has been observed to be mainly controlled by sorption reactions to clay particles, organic compounds and Fe/Mn oxyhydroxides rather than precipitation mechanisms (Farrah *et al.* 1980; Christensen 1984). The sorption of Cd has been demonstrated to be mainly controlled by pH, with a 3-5 fold increase in sorption strength with each increasing pH unit (Christensen 1984; Bermond and Bourgeois 1992; Boekhold and Van der Zee 1992), with soil texture and organic matter content reported to have a smaller impact on Cd solubility (Boekhold and Van der Zee 1992; Degryse *et al.* 2009). Aging effects and resident time in the soil environment does not have a substantial effect on Cd sorption. Therefore, most Cd that is initially bound when it enters the soil system will not increase or decrease with time, although due to the labile nature of Cd^{2+} , sorption and desorption can change depending on environmental conditions (Christensen 1984; Hamon *et al.* 1998; Seuntjens *et al.* 2001). Additionally, Cd solubility is not regarded to be significantly influenced by the addition of organic matter unless the initial concentration of soil OM is very low due to the limited affinity of Cd^{2+} for organic complexation (Bergkvist *et al.* 2005; McLaughlin *et al.* 2006; Degryse *et al.* 2009). Therefore, adding organic matter to remediate Cd contaminated soils is less effective than for elements that participate strongly in organo-metallic complexation (such as Pb and Cu).

2.6 Remediation Approaches

Several remediation approaches to solve the issues of Pb/Zn mine impacts have been developed, ranging from conventional engineering techniques to novel reclamation methods. The effectiveness of these techniques varies considerably depending on the physical, chemical and topographical nature of the site in

question, leading to uncertainties in their application and generalisable use as remediation tools.

The costs of remediation can differ substantially and the treatment of Pb contaminated land can be expensive (Table 2-1). This can be prohibitive to private landowners, such as is the case at the Tyndrum and Le Bleymard Pb/Zn mines, where budgets for remedial actions would be minimal. Therefore, the scientific community is consistently searching for viable alternative methods for the alleviation of pollution resulting from Pb/Zn mines. A brief critique of remedial approaches are discussed in the following sections.

Table 2-1. Comparative costs of remediation technologies (£). Conversions of USD to GBP based on an exchange rate of 1.00 USD = 0.657 GBP. CL:AIRE variability band states the standard deviation of the range of remediation costs collected in the study and the variability of price within that technology. Insufficient data due to low response rate in the study (CL:AIRE 2010; FRTR 2014).

Technology	FRTR Cost Range (£m³)	CL:AIRE Cost Range (£m³)	CL:AIRE Variability Band (£m³)
Soil washing	46 - 123	25 - 60	40 - 80
Phytoremediation	97 - 1529	5 - 50	Insufficient data
Soil Flushing	15 - 42	25 - 60	10 - 40
Immobilisation	33 - 217	60 - 100	40 - 80
In situ thermal treatment	25 - 53	60 - 100	40 - 80
In Situ Enhanced Bioremediation	20 - 66	25 - 60	10 - 40
Chemical Oxidation/Reduction	125 - 434	25 - 60	40 - 80
Ex situ Thermal Treatment	29 - 166	>100	>80

2.6.1 Conventional Remediation

Conventional remediation approaches mainly focus on the physical or chemical treatment of the mine tailings. Physical treatment utilising clean soils as a diluent is a common approach in large scale remediation efforts, where imported soils are mixed into the tailing material to reduce the total elemental concentrations. This has been shown to be immediately effective in reducing the health risks to local communities (Mielke *et al.* 2016, 2017; Laidlaw *et al.* 2017), improving soil quality and reducing the concentrations of PTE, particularly when the contaminated soil is buried beneath the imported material (Douay *et al.* 2008; Khalid *et al.* 2016). Similarly, capping approaches and the use of geotextiles and materials exhibiting low permeability have been extensively used in conventional remediation efforts and again have been found to reduce the impacts of Pb to human health (Mielke *et al.* 2011). This is achieved by placing a physical barrier over the contaminated material and substantially reducing the risk of plant absorption and human interaction (Ericson 2014; Ericson and Dowling 2016). Additionally, these systems can also incorporate sub-surface treatment barriers to address continued pollution from groundwater movement, with the barriers comprising of low permeability materials (e.g. clays) geotextiles (polyester sheets) or reactive membranes (zerovalent iron, limestone) inducing the precipitation and immobilisation of the targeted pollutant (Blowes *et al.* 2000; Mulligan *et al.* 2001a; Thiruvengkatachari *et al.* 2008). The associated costs of these approaches will depend on the environment and materials used but may range between £120 – 500 per m² (CL:AIRE 2010).

Traditional chemical treatment efforts include the use of soil washing, chemical encapsulation and electrokinetic remediation. Soil washing is an ex-situ procedure that involves the use of an extractant solution such as chelating or desorption based chemicals to remove Pb from the contaminated substrate (Table 2-2) (Dermont *et al.* 2008; Jiang *et al.* 2011; Yang *et al.* 2012). The washing process involves a physical agitation over a quantified period, allowing the transfer of the target element from the solid to the aqueous phase. Soil washing has been identified as an effective method in the removal of Pb from mine soils (Moutsatsou *et al.* 2006; Yang *et al.* 2009; Kim and Kim 2011) due

to the simultaneous effects of removing the clay fractions of the soils (<0.002 mm) and the desorption of Pb from the solid phase across several fractions, depending on the reagent applied (CL:AIRE 2007). The processed material can often be returned to the excavated site or disposed as inert material at a cost of approximately 2/3 as compared to containment via a hazardous landfill facility (WRAP 2013).

Chemical encapsulation refers to the chemical and physical immobilisation of PTE via the application of other materials, often with a pH of greater than 8.5 to promote the formation of insoluble species as well as concurrently producing a physical binding mechanism (Table 2-2) (Chen *et al.* 2009; Choi *et al.* 2009; Voglar and Lestan 2010; Desogus *et al.* 2013). This material can then be applied as road construction materials or as inert backfill depending on the structural properties and the leaching risks assessed from the final product.

Table 2-2. Example chemicals and materials used in soil washing and chemical immobilisation remediation techniques.

Remedial Approach	Examples	Reference
Soil Washing		
Chelants	EDTA, DTPA, oxalate, citric acid	(Steele and Pichtef 1998; Dermont <i>et al.</i> 2008)
Other complexation compounds	Surfactants, sodium metabisulfite	(Abumaizar and Smith 1999; Neilson <i>et al.</i> 2003)
Desorbants	HNO ₃ , HCl, H ₂ SO ₄ , NaCl, CaCl ₂ , H ₃ PO ₄	(VanBenschoten <i>et al.</i> 1997; Lim <i>et al.</i> 2004; Ko <i>et al.</i> 2005)
Immobilisation		
Chemical Encapsulation and immobilisation	Cement, limestone, zeolites, charcoal, fly ashes	(Shende <i>et al.</i> 1994; Dermatas and Meng 2003; Fawzy 2008; Chen <i>et al.</i> 2009; Choi <i>et al.</i> 2009; Voglar and Lestan 2010; Desogus <i>et al.</i> 2013; Leelarungroj <i>et al.</i> 2018)

Electrokinetic remediation is based on the migration and collection of PTEs via electrical fields when a material is saturated in a conductive solution. Across the treatment area cathodes and anodes are installed and target PTEs migrate towards the oppositely charged field, with electrophoresis, and electro-migration allowing movement of dissolved PTE and those bound in organic complexes (Khalid *et al.* 2016). The technique is often enhanced by the use of chelants such as EDTA or desorption agents (e.g. HCl, citric acid) to increase the aqueous phase concentration of PTE and improve the conductive properties of the saturated material, although consideration on the choice of reagent is key if multiple elements are to be extracted (Suzuki *et al.* 2014; Vocciante *et al.* 2016).

Unfortunately, despite the high rate of PTE removal, hard conventional systems can exacerbate soil degradation and reduce soil quality, negating the remediation process. Electrokinetic based strategies often raise soil pH and inhibit microbial and enzyme functioning, reducing the ability of micro-faunal and plant re-establishment without further amendments and post-treatment care (Lear *et al.* 2007; Cang *et al.* 2012; Pazos *et al.* 2012). Soil flushing and washing have been criticised for the associated high energy and consumable inputs and the potential risks of pollution events from residual additives and leachates (Atteia *et al.* 2013; Kim *et al.* 2013; Jelusic and Lestan 2014). Overall, although these processes produce reliable and prompt metal decontamination solutions they can produce new soil pollution issues that require attention, limiting their usefulness on sites which may be ecologically sensitive.

2.6.2 Ecological Remediation Approaches

In the last two decades the scientific community have investigated alternative remediation approaches, aiming to identify cheaper and less environmentally damaging methodologies to remediating abandoned mine tailings. A common constituent in this approach is to exploit and enhance biotic processes that can sequester, remove or otherwise reduce the risk of PTE impacts to human health and the wider environment (Gadd 2010; Wu *et al.* 2010; Gaur *et al.* 2014). The main rationale behind this is to allow self-sustaining biological mechanisms to replace expensive engineering solutions that require less input of resources and

labour. Additionally, there has been substantial interest in the use of inorganic and organic waste materials to substitute the traditional utilisation of clean soils or cements to reduce PTE impacts, with the parallel benefits of avoiding costly disposal to landfill of these materials and the improvement of contaminated lands. The main technologies used in ecological approaches for the remediation of Pb/Zn mine tailings can be classed under the broad definitions of organic amendment incorporation, phytoremediation and microbial sequestration.

2.6.2.1 Organic and Inorganic Amendment Incorporation

Several inorganic and organic waste materials have been found to have a range of effects on the chemical behaviour, physical structure and biological functioning of PTEs when applied to mine tailings. The primary mechanism in this remedial approach is the immobilisation of PTEs via adsorption and complexation onto chemically and kinetically stable surfaces, either as inorganic or organic components of the material or via precipitation of labile PTE into insoluble species (Park *et al.* 2011).

Adsorption has been described as the accretion of a target element or compound onto a solid phase, which may be either a product of electrical attraction between the adsorbent and the chemical in question or via direct chemical bonding (Alloway 2012). The phenomena of adsorption is heavily influenced by the presence of clays, Al/Fe/Mn oxy-hydroxides and organic matter (Kumpiene *et al.* 2008; Merdy *et al.* 2009). Furthermore this process is also significantly regulated by pH (Lee *et al.* 1998; García-Sánchez *et al.* 1999), with increasing pH producing a net increase in negative charge in the soil environment, thus promoting a greater surface attraction for common PTE cations (e.g. Pb^{2+} , Cu^{2+} , Zn^{2+} and Cd^{2+}).

Precipitation of Pb has been known to be an effective management tool, where mobile species are converted to less soluble forms, e.g. $\text{Pb}(\text{NO}_3)_2 \rightarrow \text{Pb}_3(\text{PO}_4)_2$ by the addition of anions such as phosphate, carbonate and sulfate (Adriano 2001). With specific reference to Pb, the precipitation of labile Pb^{2+} has been achieved using P-rich compounds, such as phosphate fertilisers (Gupta *et al.* 2014), phosphoric acid (Cao *et al.* 2009) and bone meal (Chrysochoou *et al.*

2007). The formation of $\text{Pb}_3(\text{PO}_4)_2$ generates a compound with a solubility product of -11.43 , enabling a highly stable Pb compound that in most studies reduces the environmental risk of Pb to soil biota and human health (Zeng *et al.* 2017).

Several organic materials have been applied to remediate Pb/Zn mine tailings. For example, sewage sludge has been incorporated into Pb/Zn tailings and found to reduce DPTA-extractable Pb and Zn (Chiu *et al.* 2006), promote the formation of insoluble Pb species (Andrés and Francisco 2008) and reduce soluble Pb and Zn over a three year period (Ciarkowska *et al.* 2017). Similarly, other materials such as biochar, lime, compost, cockle shells and pig slurry have been observed to reduce labile PTE concentrations due to increasing tailing pH, promoting stable complexes and stimulating the formation of insoluble PTE species, leading to a reduced impact to plants (Ye *et al.* 2000; Zornoza *et al.* 2013; Puga, Abreu, Melo and Beesley 2015; Puga, Abreu, Melo, Paz-Ferreiro, *et al.* 2015; Forján *et al.* 2016; Islam *et al.* 2017). It has been suggested that materials containing more condensed and humified organic binding sites are preferred as these are less likely to release previously immobilised PTE into more labile fractions (Chotpantarat *et al.* 2015). There may also be evidence that organically held PTE inhibit bio-degradation and therefore reduce the risk of metabolisation and subsequent release as more labile compounds (Merckx *et al.* 2001). This would be beneficial to increase the longevity of a remediation process and limit the potential for release of bound PTE in the future.

However, organic inputs have also been observed to increase the concentration of labile PTEs due to the introduction or formation of water soluble carbon (WSC), promoting PTE transfer from the solid phase into a more mobile form (Romero *et al.* 2005; Clemente and Bernal 2006; Hanč *et al.* 2008; Beesley *et al.* 2014a). Other complications can arise if organic amendments contain high concentrations of organic acids, reducing the pH and therefore adsorption capacities and introducing chelatants to the remediated system (Singh and Agrawal 2007, 2010). Furthermore, the choice of organic amendment can influence or increase the presence and mobility of specific PTE in a soil matrix,

for example, poultry litter and biochar applications to soils have been found to increase the total concentration and mobility of As due to an increase in pH caused by the desorption of the As anion by hydroxide ions (Rutherford *et al.* 2003; Beiyuan *et al.* 2017; Lomaglio *et al.* 2017). Therefore, careful consideration of the organic amendment and the target PTE is essential before undertaking remedial action on contaminated soils or mine tailings.

2.6.2.2 Phytoremediation

Phytoremediation is the use of vascular plants and associated microbes to reduce the environmental impacts of pollutants in a contaminated site, either by contaminant removal, immobilisation or degradation into less harmful substances (Vamerali *et al.* 2010). With reference to sites contaminated by PTE, phytoremediation falls into two broad categories: phytostabilisation, where PTE enriched material is prevented from further movement into the wider environment, and phytoextraction, where PTEs are accumulated in the aerial compartments of plants which can then be harvested and removed from the impacted soil over time (Raskin and Ensley 1999).

As mentioned, phytostabilisation is the use of vegetation to physically reduce the movement of contaminated soil/tailings and in some cases reduce the chemical mobility of the target PTE. This is generally achieved through the introduction of a dense rooting system and a vegetative cover to limit the effects of raindrop and sheet erosion processes, reducing the likelihood of rill and gully formation which may exacerbate a further loss of material (Boisson *et al.* 2005; Radziemska *et al.* 2018). Furthermore this approach can also aid in the retardation of solubilised PTE in the soil solution by absorption and adsorption via roots (Alkorta *et al.* 2010; Bolan *et al.* 2011). Phytostabilisation aims to minimise translocation of PTE from the roots to the shoots to reduce eco-toxicity risks and the subsequent movement of PTE in the food chain, and so the focus is on plant species that are ‘excluders’ of PTEs.

Several species have been identified as useful phytostabilisation candidates for Pb, Zn and Cd. For example, Hook. (*Athyrium wardii*), Sabiá (*Mimosa caesalpiniaefolia*) and Tall fescue (*Festuca arundinacea*) (Ribeiro de Souza *et*

al. 2012; Zou *et al.* 2012; Hu *et al.* 2015) have been identified as limiting Pb translocation from the root to the shoot system whilst tolerating high Pb burdens, Welsh polypody (*Polypodium cambricum*) and Red spire (*Melica transsilvanica*) (Roccotiello *et al.* 2010; Turnau *et al.* 2010) for Zn phytostabilisation purposes, and White lupin (*Lupinus albus*) and Pearl millet (*Pennisetum americanum*) when considering Cd phytostabilisation (Vázquez *et al.* 2006; Zhang, Xia, *et al.* 2010). Several of these species were observed to be multi-elemental tolerant, providing evidence of a useful species for phytostabilisation.

Phytostabilisation has been demonstrated to be effective in the remediation of Pb/Zn tailings, usually in combination with organic amendments e.g. (Lei and Duan 2008; Galende, Becerril, Barrutia, *et al.* 2014; Lee *et al.* 2014; Gil-Loaiza *et al.* 2016; Garaiyurrebaso *et al.* 2017; Pardo *et al.* 2017a), where the combined use of organic material and PTE tolerant species has allowed the establishment of a vegetative system, and often the reduction in PTE mobility. It is clear therefore, that phytostabilisation has strong supporting evidence as a viable option for the remediation of Pb/Zn mining landscapes.

Contrastingly, phytoextraction often aims to promote the mobility of PTE in the soil/tailing environment to allow plant uptake of the target element. With this technology, plant species within the *Brassica* family, such as *N.caerulescens* (Zhao *et al.* 2003; Hammond *et al.* 2006; Purakayastha *et al.* 2008; Kaur *et al.* 2012), termed hyperaccumulators are often used in conjunction with chelating compounds e.g. (Saifullah *et al.* 2009; Bhargava *et al.* 2012; González *et al.* 2014) to provoke greater bioavailability, plant uptake and translocation of PTE from the root system to the aerial parts of the plant. Several hyperaccumulating species have been found for Cd and Zn, such as the gelonium poison-leaf (*Dichapetalum gelonioides*), (*Sedum alfredii*, no common name) and (*Picris divaricate Vant.*, no common name) (Yang *et al.* 2004; Tian *et al.* 2011; Broadhurst *et al.* 2013; Nkrumah *et al.* 2018). Few species have been identified as potential Pb hyperaccumulators, such as Indian mustard (*Brassica juncea L*) and Rattlebox (*Sesbania drummondii*), however the hyperaccumulation

potential of these plants have only been demonstrated in hydroponic or agar based matrixes and soluble Pb-salts, with no current evidence of Pb hyperaccumulation in soil systems (Liu *et al.* 2000; Sahi *et al.* 2002; Ryuji Takeda, Yukiko Sato, Rumi Yoshimura and Sawabe 2010). This is in contrast to the numerous identification of Cd/Zn identified species, and is generally attributed to the analogous nature of Cd and Zn and the use of Zn biochemical pathways for the enhanced uptake of Cd (Yang *et al.* 2005a; Verbruggen *et al.* 2009a, 2009b).

Regarding phytoextraction, the technology has not been generally viable for the removal of PTE in mine tailings. Reviews by Mendez and Maier, (2008) and by Ernst, (2005) found that promising results from greenhouse trials did not transpose well into field studies, with the several impediments such as low biomass production, lengthy remedial periods and a limited range of extraction depth due to shallow roots. Furthermore, a poor uptake in the use of this technology in industrial remediation efforts suggests a lack of confidence in phytoextraction for highly contaminated lands, possibly due to the uncertainties in economic costs and effectiveness (Vangronsveld *et al.* 2009; Robinson *et al.* 2015). However, research into the phytoextraction of mine tailings continues (Krisnayanti *et al.* 2016; Alcantara *et al.* 2017; González-Valdez *et al.* 2018; Pardo *et al.* 2018), with a new focus on the recovery of rare earth elements, which may indirectly improve environmental quality by improving soil conditions, cover the costs of implementing a remediation system via phytomining and establishing a protective vegetative layer similar to phytostabilisation.

Overall it is evident that most phytoextraction processes are not currently viable as a remedial approach for mine tailings, unless it is possible to substantially accelerate the accumulation process of biomass yield of the vegetation used. Conversely, phytostabilisation displays merits in the alleviation of environmental impacts from tailing heaps, although few large-scale trials exist.

Literature surrounding the regeneration of mine tailings often cite PTE concentrations as a factor inhibiting vegetative growth, rather than the overarching cause of vegetative establishment. For example, Courtney, (2013)

observed that although the concentrations of Pb and Zn were restricting vegetative establishment, salinity, pH and lack of nitrogen (N), phosphorous (P) and potassium (K) were also factors that resulted in limited plant cover. Similarly Rodríguez-Seijo *et al.*, (2014) found that the pervasive stoniness and shallow soils of a Pb/Zn mine were a key factor in the failure of vegetative establishment, as was the low organic matter content ($11.1 \pm 5.4 \%$) and poor cation exchange capacity ($9.9 \pm 6.1 \text{ cmol kg}^{-1}$) of the soil. The author stated that although Pb and Zn concentrations were a significant problem, an overall balance in all the soil components needs to be met to attain a functioning soil system. This was mirrored in work by Sélack *et al.*, (2013) who observed that PTE content was only a part of the issue and highlighted that restoration must look at all aspects of the site and the surrounding environment for long lasting remediation success.

In most studies the authors have emphasised the need for organic amendments as mine tailings are typically low in essential plant nutrients (N,P,K) and organic matter *e.g.* (Zornoza *et al.* 2012; Araújo and Costa 2013). Further emphasis on the incorporation of organic material and particularly those which are viewed as wastes has been a recent interest throughout the literature. This allows the diversion of wastes from landfill sites, and has included sludges, animal manures, and organic wastes sources from households and industry (*e.g.* marble wastes, horticultural waste compost and municipal food waste) (Means *et al.* 2005; Li *et al.* 2006; Melgar-Ramírez *et al.* 2012; Kabas *et al.* 2014).

Whilst the PTE found in tailing heaps are a key remediation target, thought must also be given to other components in the tailing system. To provide a habitat that can support a range of plant and animal species a suitable soil must be created that not only has acceptable concentrations of PTEs but also meets the structural and biochemical needs of the ecosystem surrounding it.

2.7 Conclusions

From the review of the current literature mining activities and the legacies left from pre-legislative operations have resulted in substantial pollution of the environment. The abandoned tailings at Tyndrum and Le Bleymard have been identified as key sources of pollution within their respective environments and

continue to pose a risk to environmental and human health. Unfortunately, remedial efforts have not been undertaken at either site, potentially due to economic reasons, or due to a lack of technical knowledge surrounding the possible approaches that could be adapted. From this chapter phytostabilisation and the incorporation of organic amendments may be viable options for remediation, however a comprehensive understanding of each of the sites and the implications of applying either remedial approaches must be further understood before generalisations can be made.

Chapter 3. Methodology

3.1 Introduction

To achieve the objectives and address the overall project aims detailed in Chapter 1 a series of linked laboratory and field studies were conducted to inform current literature and provide the basis of final field experimental design.

The purpose of this chapter is to introduce the methodologies applied during sampling procedures, experimental design and analytical approaches adopted during the project. Furthermore, experimental conditions, sampling regimes, handling and storage of samples and an overview of the instruments and chemical analyses that were applied throughout the investigation is provided.

3.2 Site Characterisation

3.2.1 Overview

Site characterisations of both mining sites were undertaken to ascertain the concentration and distribution of selected PTEs, pH, TN, TC and PS. From both locations, soil samples were obtained and both vegetation coverage and species identified. Geo-spatial approaches, discussed in section 3.2.3 were undertaken to allow for mapping of PTE, pH TN and TC which could then be used to inform future decisions regarding field trials and to identify areas of interest.

3.2.2 Sampling Strategy

Tailing Material

At each defined GPS location, detailed in section 3.2.4, a composite tailing sample from 0 – 5 and 5 – 20 cm depth was recovered using a pre-cleaned soil auger (50 cm maximum depth, soil core diameter = 30 mm) and placed into a clean, labelled polyethylene bag. Composite samples were created by taking three individual tailing samples in a triangular formation approximately 10 cm distance apart and combining the respective depths in the same bag to produce a sample of approximately 200 g of the tailing material.

Vegetation

Vegetation was assessed by randomly placing three 50 cm² quadrats at each GPS location, within 1 m of the defined point. Vegetation coverage was assessed

following the NVC for total vegetation cover (Rodwell 2006), and species inside the quadrats were identified. No vegetation was removed from the site.

3.2.3 Inverse Distance Weighting, Kriging and Empirical Bayesian Kriging

Inverse distance weighting (IDW) is a simple, non-linear interpolation approach that applies a weighted average from nearby known points to predict an unknown value. The prediction is based on the proximity of known neighbour samples and an assigned *power value* which describes the influence of proximity to the predicted point (Equation 1). With increasing power values the influence of a known point increases with closeness to the predicted point. Therefore, higher power values may be required where high spatial heterogeneity exists.

Inverse distance weighting is useful for data which does not conform to convention distributions and can give straightforward outputs, however there is no definition of error in the model, leading to uncertainties in the accuracy of the analysis.

$$\hat{V}_1 = \frac{\sum_{i=1}^n \frac{1}{d^p} v_i}{\sum_{i=1}^n \frac{1}{d^p}}$$

Equation 1. Formula for IDW interpolation. Where: V = value estimated, V_i = known value, d = distances from n to known data points to the power of p.

Kriging is a similar interpolation approach, with the distinction that the model assumes that the distance and the direction between known locations construes a spatial relationship which can then be used to estimate variability. The model is multifaceted and determines the distribution of the data, produces a semivariogram and attempts to produce a statistical model that can explain the predictive 2D surface, alongside the error associated with this process (Equation 2). Kriging is often the preferred geo-statistical method due to the ability to avoid issues with bunching of known data points and the resulting errors when attempting to estimate unknown locations (Mosammam 2013).

$$\hat{Z}(s_0) = \sum_{i=1}^N \lambda_i Z(s_i)$$

Equation 2. Simple kriging. Where: $Z_{(si)}$ = the measured value at the i th location, λ_i = an unknown weight for the measure value at the i th value, s_0 = the prediction location, N = the number of measured values.

Empirical Bayesian kriging (EBK) differs from the standard approach by accounting for the error inherent in the semivariogram produced in the kriging process, aiding in avoiding underestimation in the predictive model. The general advantages of EBK are less interactive modelling, greater accuracy in prediction and a usefulness for small datasets.

Empirical Bayesian kriging and IDW were used to produce prediction models of spatial PTE and other soil characteristic distributions. Inverse distance weighting was used to produce simple visual descriptions of the previously mentioned distributions in the Tyndrum site as modelling approaches using EBK were not appropriate. This was due to a lack of spatial correlation and direction in the concentration of PTE, which was a fundamental assumption of the kriging approach. Further information on this is found in Chapter 4. EBK was applied to predict concentrations at the Le Bleyard mine, and to determine the error in the overall spatial projections used in this study area. Both techniques were completed using ArcGIS ver. 10.1.

Kriging models were based on the 0-5 cm sampling depth to describe the concentration of PTEs that will immediately affect plant germination and be subject to surface environmental conditions, such as wind and rain erosion.

3.2.4 Kriging Sampling Strategy

Tyndrum

ArcGIS ver. 10.1 and the Sampling Design Tool (Buja 2012) was used to produce a stratified random sampling regime (Figure 3-1) prior to sample collection. The sampling area was confined to the main tailing area and excluded surrounding forestry cover. Due to time and budget constraints a total of 24

locations were determined per site, giving a sampling cover of approximately 1 sample per 470 m².

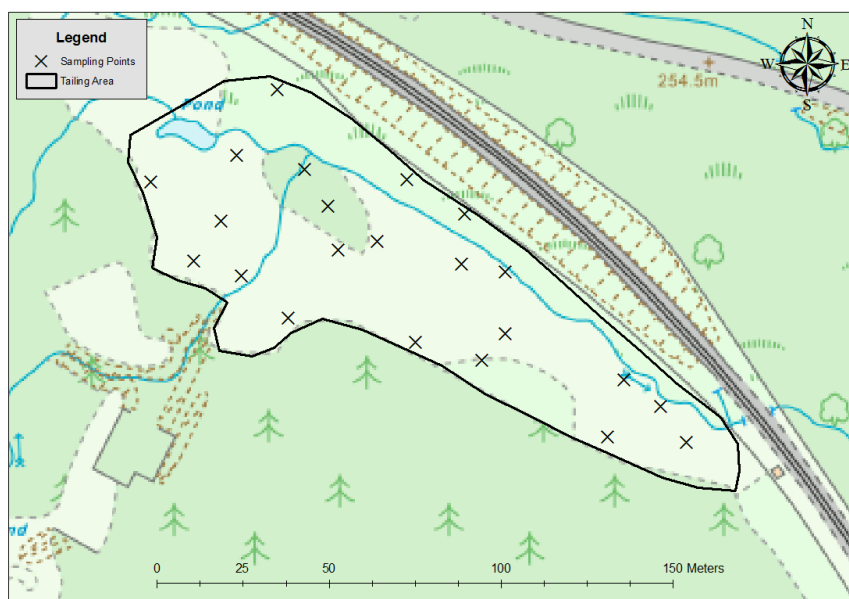


Figure 3-1. Stratified random sampling locations at the Tyndrum Pb/Zn mine.

Samples were collected in May 2015 using a Garmin eTrex[®] 10 (acc. ± 1.5 m) to locate each point.

Le Bleymard

The sampling strategy for Tyndrum was replicated at the tailings in Le Bleymard (Figure 3-2). As the mine area in Le Bleymard is considerable larger, a sampling cover of roughly 1 sample per 1,500 m² was obtained.

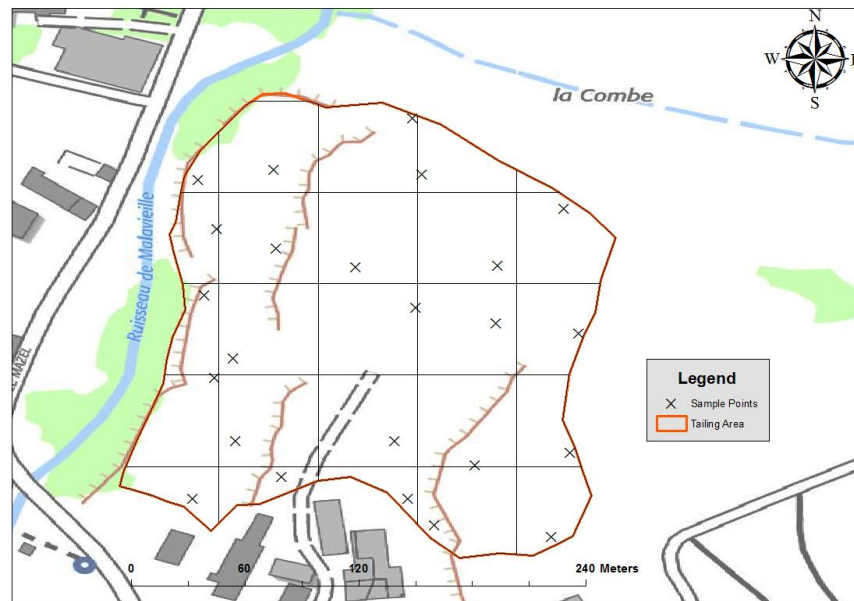


Figure 3-2. Stratified random sampling locations at the Le Bleymard Pb/Zn mine.

Accuracy of Predictive Modelling

The accuracy of spatial prediction using EBK and IDW were verified using 5 random samples collected from each mine that were not included within the spatial model, therefore acting as an independent standard. For Le Bleymard, the random samples were found to fall within the expected error range for each modelled location, therefore validating the predictive approach. For Tyndrum as IDW does not provide error measurements, the mapping is used to convey heterogeneity of the site only.

3.2.5 Water Sampling - Ruisseau de Malavieille

The Ruisseau de Malavieille (Brook of Malavieille) originates from the upper areas of Mont Lozère and flows past the mine at Le Bleymard before joining with le Lot approximately 2 km downstream from the tailing area (Figure 3-3).

From the Ruisseau de Malavieille 12 duplicated samples were taken in the middle of the stream at an approximate depth of 10 cm in April 2016, April 2017 and October 2017 using 50 ml centrifuge tubes, rinsing the tubes out three times with river sample before overfilling the tube with sample to ensure that no headspace is present. Samples were immediately filtered via a 0.22 μm PTFE syringe filter (Merck Millipore, Germany) into a clean 50 ml centrifuge tube. A

Garmin eTrex® 10 GPS unit (acc. ± 1.2 m) was used to record each sampling point. The water samples were placed in a cool box and subsequently assessed for pH (section 3.72) and total PTE content (section 3.7.18). The water samples were taken to support the characterisation of the tailings as no previous data were available at this site.

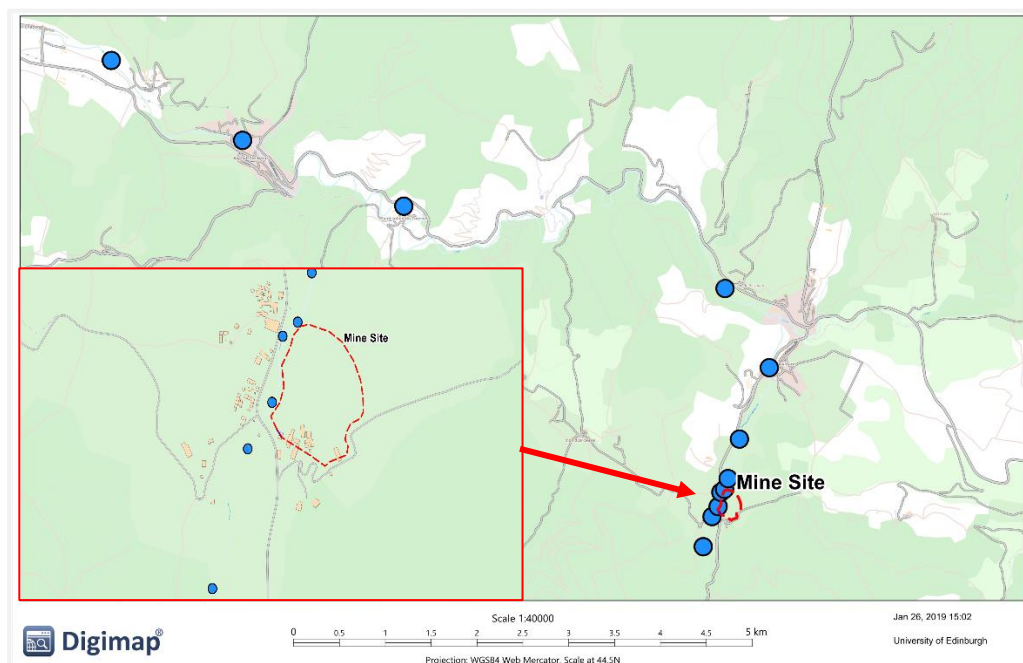


Figure 3-3. Water sampling locations along the Ruisseau de Malavieille.

3.2.6 Water Sampling - Unnamed Tyndrum River.

A single set of water samples were taken from the Tyndrum tailings in May 2015 (Figure 3-4) and were not repeated due to similarities with extensive previous studies on the water column concentrations of PTE (Mansor 2008). Water samples were handled following section 3.2.5.

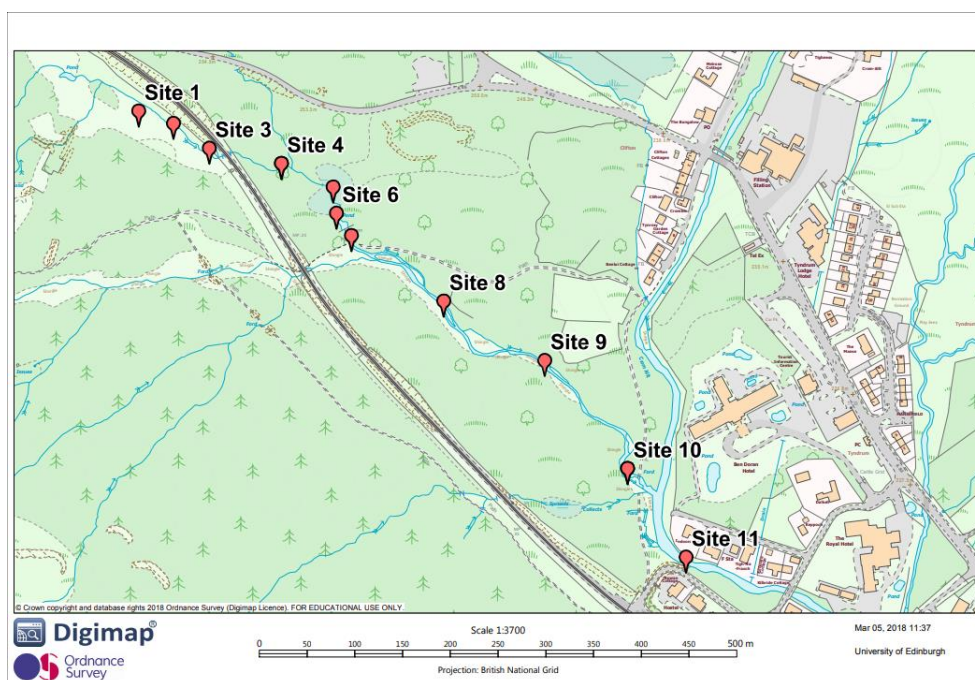


Figure 3-4. Water sampling locations at the unnamed watercourse near the Tyndrum Pb/Zn mine.

3.3 Phytotoxicity Investigation

3.3.1 Overview and Rationale

Based on vegetation surveys from Tyndrum and Le Bleymard it was clear that a range of species have naturally colonised areas of the mine tailings. It is useful to screen several species for their potential in revegetation efforts on site as part of the field trials described later in this chapter. This should initially be undertaken in a controlled environment to inform future field trials by confirming responses of species to PTE burdens to maximise revegetation outputs in field studies. Furthermore, it is essential to ascertain whether the species display characteristics of phytoextraction and/or phytostabilisation candidates, with the latter being preferential in the aims of this study (section 2.6.2.2). Finally, it is important that the experimental conditions reflect the environmental situation that are encountered at both sites, so that reasonable projections can be made and future experiments can be reliably informed.

3.3.2 Overall Aims

The overall aim of the phytotoxicity experiment is to attempt to identify local plant species that can be used in the restoration of the Pb/Zn mines located in Tyndrum and Le Bleymard. It is envisaged that some species may show promise for PTE accumulation, or at least a degree of tolerance for surviving in PTE enriched sites and supporting tailing stabilisation.

The results from these glasshouse experiments should identify tolerant plant species that will be used within the field trials located on the abandoned Pb/Zn mines, where the effectiveness of phytoremediation will be further explored.

This investigation consisted of an initial screening of eight key species found at Tyndrum and Le Bleymard and a secondary experiment focusing on the uptake, translocation and growth restriction of the species that showed the most promise from the initial study when subjected to concentrations of labile Pb and Zn projected to be found at Tyndrum and Le Bleymard.

3.3.3 Objectives

1. To design an experiment that represents the physical and chemical constraints of the mine tailings in a simulated tailing environment.
2. To identify the most successful species in terms of growth and resilience to the chemical constraints of the replicated tailing environment.
3. To assess whether species are potential phytostabilisation candidates.
4. To identify species that can be used in future field experiments.

3.3.4 Research Questions

The following questions are derived from the aims and objectives:

1. Is there one specific plant species that displays significantly greater resilience to PTE burdens encountered in the contaminated tailings at each site in relation to the site and tailing characteristics?

Hypothesis 1

H₀: There are no significant differences between species in terms of tolerance to encountered PTE burdens.

H₁: There are significant differences between species in terms of tolerance to encountered PTE burdens.

2. Do species exhibit evidence of hyperaccumulation or interaction (for instance, uptake, toxicity, avoidance etc.) of encountered PTE?

Hypothesis 2

H₀: Tested species do not display qualities of PTE hyperaccumulation.

H₁: Tested species do display qualities of PTE hyperaccumulation.

3. What factors or variables will need to be considered when conducting field trials which may be inherently more complex?

3.3.5 Species Considered

The plant species studied have either been observed to grow on the Pb/Zn mines studied or within the greater geographical region (within 10 km²). No non-native or invasive species were selected to reduce issues related to biodiversity and local cultural values. Some species are well reported to exhibit tolerance or accumulative behaviour of PTEs (not necessarily Pb or Zn), whilst others, to the best of the author's knowledge, have not previously been investigated for their potential role in phytoremediation. A brief introduction and rationale behind each plant species are provided as well as a table for comparison of species (Table 3-1).

Table 3-1. Presence of phase 1 tested species at Tyndrum or Le Bleymard

Species	Le Bleymard	Tyndrum
<i>Capsella bursa-pastoris</i> (Shepherd's purse)	✓	✓
<i>Molinia caerulea</i> (Purple moor-grass)	✓	✗
<i>Cardamine hirsute</i> (Hairy bittercress)	✓	✗
<i>Noccaea caerulea</i> (Alpine pennycress)	✗	✓
<i>Agrostis capillaris</i> (Common bent)	✓	✓
<i>Agrostis vinealis</i> (Brown bent)	✓	✓
<i>Lavandula angustifolia</i> (Common lavender)	✗	✗
<i>Festuca ovina</i> (Sheep's fescue)	✓	✓

***Capsella bursa-pastoris* (Shepherd's purse)**

This species was observed to grow on the Le Bleymard and Tyndrum mine tailings during the site characterisation studies, and is a known Cd accumulator (Lin *et al.* 2014). The species has also been described as tolerant to Zn and Ni, and has been highlighted as a potential biomonitor of other PTEs (Aksoy *et al.* 1999; Kozhevnikova *et al.* 2014). No studies were found that considered using this species as a Pb/Zn mine tailing restoration tool. This species is also found within the wider Mont Lozère region (Parc National des Cévennes 2017).

***Molinia caerulea* (Purple moor grass)**

This plant is commonly found in Scotland, although was not identified on the Tyndrum mine itself. Studies considering this species are limited and have not recognised accumulator characteristics, although Denaix *et al.*, (2011) speculated that *M.caerulea* may be a Ni accumulator. The species has been observed to grow on contaminated land in the Peak District National Park (Badsha and Badsha 1988; Pickett 2011) and at least one Pb/Zn mine tailing site (Ryszka and Turnau 2007).

***Cardamine hirsuta* (Hairy bittercress)**

This species was identified throughout the Tyndrum site and has previously been acknowledged as a Cd accumulator (Pérez-Esteban *et al.* 2014). The species has also survived on abandoned chemical factory sites (Andreucci *et al.* 2006) and current landfill areas (Giupponi *et al.* 2014). There is no current evidence surrounding *C. hirsuta* and its tolerance to Pb or Zn.

***Noccaea caerulea* (Alpine penny-cress)**

This plant has been well documented as a Cd and Zn hyperaccumulator and is considered a model species in the study of phytoremediation (Halimaa *et al.* 2014; Mandáková *et al.* 2015). The species has been identified to extract several metals from the soil system, including Ni, Cd, Zn and under some conditions Pb (Huang and Cunningham 1996; Meerts and Van Isacker 1997; Mohtadi *et al.* 2012). This species has been known to inhabit the mine tailings within the Mont Lozère (Reeves *et al.* 2001; Peer *et al.* 2003) and grows extensively within the Le Bleymard site.

***Agrostis capillaris* (Common bent)**

This species is common throughout the Cevenne national park (France) and has been identified as a potential PTE accumulating candidate for several metals (Dekoe 1994; Pastor *et al.* 2007; Freitas *et al.* 2009). The plant is also found in the region of the Tyndrum mine, and so may provide a common candidate for both sites (Land Use Consultants 2012).

***Agrostis vinealis* (Brown bent)**

This species is common at both study sites, and has been observed to exhibit tolerances to Pb soil contamination (Baker and Proctor 1990), with one study demonstrating an accumulation of 2930 mg kg⁻¹ of Pb in the plant tissues (Pichtel and Salt 1998). Another study gives evidence for this plant as tolerant to high Mg and Ni (Nagy and Proctor 1997). However, the species has received limited attention as a potential tool for restoration of abandoned mines despite its published tolerance for high metal and low nutrient conditions.

***Lavandula angustifolia* (Common lavender)**

Common lavender is cultivated in the south of France for use in the aromatic oil and food industries with studies commenting that PTE concentrations in the essential oil bearing components of similar plants (e.g. peppermint, lemongrass) are low, despite higher accumulation in other plant tissues (Zheljazkov *et al.* 2006; Amirmoradi *et al.* 2012; Lal *et al.* 2013). Therefore, it may be possible that lavender can be grown on the Le Bleymard mine tailings and a marketable product can still be derived from the plant.

***Festuca Ovina* (Sheep's fescue)**

Sheep's fescue has been reported to grow on soils contaminated by carbonaceous Pb/Zn mine wastes (Kapusta and Sobczyk 2015; Woch *et al.* 2016) and is a species that has been attributed to be tolerant to excessive burdens of Pb, Cd and Zn. Specifically *F.ovina* has been reported to show characteristics of hyperaccumulation (Vamerali *et al.* 2010) and has been used in phytoremediation studies (Stefanowicz *et al.* 2012; Grumiaux *et al.* 2015). The species was found commonly at both Tyndrum and Le Bleymard.

3.3.6 Experimental Design: Plant Identification/Suitability Testing

Phase 1: Initial Screening

Plants were grown in a synthesised mine tailing, detailed below, reflecting the physical and chemical nature of the Tyndrum and Le Bleymard tailing substrates respectively whilst in controlled environmental conditions to identify PTE tolerant plant species. A synthetic material was created to reduce issues with the heterogeneous nature of the tailing material from both mine sites (Chapter 4) and therefore allow a consistent environment throughout the study.

Tailing Matrix

The mine tailings were replicated using sharp sand (1 mm, Diall Ltd.) and horticultural grit (5 -10 mm, Royal Horticultural Society) to imitate the physical structure of the tailing material, and GWC (John Innes, No.3) was added to simulate the organic matter content of the tailings. For Tyndrum, this gave an approximate ratio of 6:2:1 and for Le Bleymard 7:1:0.5 for sharp sand, horticultural grit and GWC respectively. Approximately 30 kg of each tailing was produced by mixing in a clean, plastic lined cement mixer (Belle, 90L capacity) at 24 rpm for 30 minutes. Concentrations of total N, P and K were 0.16 % (± 0.04), 0.03 % (± 0.01) and 0.07 % (± 0.01) respectively, which were comparable to nutrient contents found at both mine sites.

pH Adjustment

The pH of the synthesised material was adjusted using $0.1 \text{ mol L}^{-1} \text{ Al}_2(\text{SO}_4)_3$ and $0.2 \text{ mol L}^{-1} \text{ CaOH}$ to reflect the conditions of each site ($5.5 (\pm 0.5)$ and $7.5 (\pm 0.5)$ for Tyndrum and Le Bleymard respectively). 100 ml aliquots of $\text{Al}_2(\text{SO}_4)_3$ or CaOH were added to the bulked material, mixed for 30 minutes (cement mixer) and four sub-samples of the bulked matrix tested (section 3.7.2) after 3 days to allow for equilibrium. The application of the buffer solution was repeated until a stabilised (less than 0.2 fluctuation over three days) pH of the bulked material was achieved.

Spiking of Synthesised Tailing Material

Half of the artificial tailing material was spiked with Pb nitrate ($\text{Pb}(\text{NO}_3)_2$) and Zn sulphate ($\text{ZnSO}_4 \cdot 7 \text{ H}_2\text{O}$) (>99.5%, Sigma-Aldrich) dissolved in de-ionised

water. For the Tyndrum treatments, a dose of 200 and 150 mg kg⁻¹ of Pb and Zn respectively were added, and 20 mg kg⁻¹ of Zn was included in the Le Bleymard treatments to reflect the median CaCl₂ extractable concentrations derived from the results from Chapter 4. The material was again homogenised and stored in covered, plastic trays at room temperature (18 – 21°C) in the dark for 7 days. The CaCl₂ extractable (section 3.7.14) Pb and Zn concentrations in four sub samples of the bulk material were assessed after 7 days to provide an indication of metal availability to plants and the spiking process repeated with a further 50 mg kg⁻¹ of Pb and 80 mg kg⁻¹ Zn for the Tyndrum matrix to compensate for possible adsorption of these elements onto the organic components of the synthesised tailing matrix and the subsequent immobilisation. CaCl₂ extractions were used as they have often been described as a useful generalisable indicator of PTE availability (Houba *et al.*, 2000). The concentrations of CaCl₂-extractable Pb and Zn and pH of the synthesised material was checked a final time before seedlings were sown. An un-spiked control with three replicates of each species in each treatment was used to determine if differences were due to PTE concentrations or the unfavourable growth conditions of the media.

Species Allocation, Treatment Structure and Plant Growth

Plant species were allocated to their respective synthesised tailing matrix based on their presence at Tyndrum or Le Bleymard (Table 3-2).

Table 3-2. Allocation of plant species to treatment groups in the phase 1 phytotoxicity experiment.

Tyndrum	Le Bleymard
<i>Agrostis capillaris</i>	<i>Agrostis capillaris</i>
<i>Agrostis vinealis</i>	<i>Agrostis vinealis</i>
<i>Capsella bursa-pastoris</i>	<i>Capsella bursa-pastoris</i>
<i>Festuca Ovina</i>	<i>Festuca Ovina</i>
<i>Cardamine hirsuta</i>	<i>Lavandula angustifolia</i>
<i>Molinia caerulea</i>	<i>Noccaea caerulea</i>

For each sample approximately 500g of the relevant synthesised tailing material was placed in a 7.6 L⁻¹ plastic pot (ScotPlants Direct) and approximately 10 g of seeds were sown and covered with a fine layer (< 1mm) of the relevant tailing material. The samples were watered using tap water and placed in a glasshouse using a totally randomised design produced using Genstat V.16 and re-configured every 7 days to ensure equal lighting conditions. The samples were grown in the glasshouse for 90 days under a 16-hr daylight regime using natural light and at 21°C (± 3) which are comparable conditions to that found at both Le Bleymard and Tyndrum in the spring/summer months. Plants were watered every 2-3 days. Five replicates were assessed for each treatment, with a total of 80 plants overall in phase 1. Table 3-3 summarises the treatments in Phase 1.

Table 3-3. Summary of the Phase 1 phytotoxicity experiment, n = 60.

Species	pH		Pb (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
	5.5	7.5	200	0	150	20
<i>Agrostis capillaris</i>	✓	✓	✓	✓	✓	✓
<i>Agrostis vinealis</i>	✓	✓	✓	✓	✓	✓
<i>Capsella bursa-pastoris</i>	✓	✓	✓	✓	✓	✓
<i>Cardamine hirsuta</i>	✓	✗	✓	✗	✓	✗
<i>Festuca Ovina</i>	✓	✓	✓	✓	✓	✓
<i>Lavandula angustifolia</i>	✗	✓	✗	✓	✗	✓
<i>Molinia caerulea</i>	✓	✗	✓	✗	✓	✗
<i>Noccaea caerulea</i>	✗	✓	✗	✓	✗	✓

Harvesting of Plants

After 90 days the plants were carefully removed from the growth media by holding by the stem and removing the soil by gently shaking. Both above and below ground biomass was collected by cutting above the root system and processed following section 3.7.1 for biomass determination.

3.3.7 Phase 2: Plant uptake of PTE

Experimental design

From the initial screening, two species that demonstrated promise (*F.ovina* and *A.capillaris*) (section 5.4.3) were selected to assess Pb and Zn uptake from a contaminated media, the translocation of these elements from roots to shoots and the effect of these PTE on plant biomass.

Growth Media Preparation and Pb/Zn Spiking

Green waste compost was selected to provide a supportive substrate for plant growth and was leached to remove excess nutrients and DOC that may alter the availability of Pb and Zn. A leached GWC matrix provided a porous, supportive structure that allowed for root growth and development without reducing Pb availability via complexation with organic matter or significant ad/absorption to other soil constituents. Therefore, this matrix provided an ideal structural environment and reduced the potential of stunted plant development arising from poor structure, which may have been a compounding factor in Phase 1. The concentrations of Pb and Zn were broadly derived from the CaCl_2 extractable Pb and Zn concentrations found at both Le Bleyard and Tyndrum, however, in this instance a higher concentration of both elements (800 mg kg^{-1}) was selected to expose the species to the upper range of estimated plant available PTE concentrations.

The growth media was prepared by autoclaving approximately 5 kg of John Innes No. 3 GWC for 2 hours at 121°C and leaching the sterilised GWC with 15 litres of tap water to remove dissolved C and other nutrients from the substrate. The GWC was dried on clean plastic sheets for 48 hours, with 2 kg of the material removed and spiked with 800 mg kg^{-1} lead nitrate ($\text{Pb}(\text{NO}_3)_2$) and zinc sulphate ($\text{ZnSO}_4 \cdot 7 \text{ H}_2\text{O}$) (>99.5%, Sigma-Aldrich) dissolved in de-ionised water. The spiked material was mixed thoroughly, covered and left to homogenise for 2 weeks in the dark at ambient temperature. A sub sample of the spiked material was taken at 3, 7 and 14 days and assessed for CaCl_2 extractable Pb and Zn.

Seed Establishment and Growth Conditions

Ten grams of seed of *F.ovina* and *A.capillaris* (Emorsgate Seeds) were sown into individual 500ml plastic pots containing the spiked material and the unspiked GWC as a control. In total, 7 replicates of spiked and control samples for each species were generated and arranged in a total random design (Genstat V.16) in a glasshouse under a 16-hour daylight regime at 21°C (\pm 3) as previously described. The plants were watered every 2-3 days with de-ionised water and grown for a total of 60 days.

Harvesting of Plants and GWC

After 60 days the plants were carefully removed from the growth media and processed following section 3.7.1 for biomass, C:N and elemental determination.

Green waste compost was assessed for CaCl₂ extractable and total elemental concentrations of Pb and Zn following sections 3.7.12, 3.7.14 and 3.7.17.

Analysis of Data

Data collected on biomass, C:N ratios and elemental concentrations were assessed via One Way Analysis of Variance (ANOVA) and Tukey family grouping using Minitab v. 18.

3.4 Organic Amendment Column Investigation

3.4.1 Overview and Rationale

From site characterisations for each site (Chap. 4) both Le Bleymard and the Tyndrum Pb/Zn mines exhibited a significant lack of chemical, physical and biological tailing properties equivalent to a ‘normally’ functioning soil environment (e.g. a woodland brown earth) and therefore have a reduced capacity to immobilise potentially toxic elements (PTEs). From a pollution point of view the mine tailing has therefore currently a low capacity to immobilise PTEs due to the lack of organic adsorption sites, which are widely recognised for their potential to strongly bind PTE such as Pb, Cu and Zn (Abumaizar and Smith 1999; Schilling and Cooper 2004; Pérez-Novo *et al.* 2008; Gustafsson *et al.* 2011). Furthermore, the skeletal tailings are susceptible to erosion and transportation through the environment due to its lack of vegetative cover and the sandy, unbound nature of the tailings.

To reduce the effects of metal toxicity to plants and soil organisms, aid in supplying adequate nutrients for plant growth (N, P, K), improve tailing structure and vegetative support, it is suspected that organic materials can be incorporated into the upper layers of tailings (Park *et al.* 2011; Branzini and Zubillaga 2012; Venegas *et al.* 2015). Importantly, if the organic materials can be sourced from local waste streams then the approach to remedial actions on the mine tailings can become more sustainable than conventional pollution alleviation methods. This can aid in supporting resource efficiency and promoting a circular economy, key factors in the EU Waste Directive 2008.

Both Pb/Zn mines are situated in National Parks and therefore require caution when attempting to introduce changes to the landscape and the surrounding local ecosystems. Whilst the use of organic materials are known to aid the rehabilitation of metal burdened sites there is also evidence that these amendments can exacerbate the issue through the introduction and mobilisation of PTEs (section 2.6.2.1). Moreover, there is a possibility of producing eutrophication problems to the watercourses surrounding the mines, through the release of nutrients (particularly N and P) (Bennett *et al.* 2001; Dodds and Smith 2016). To avoid this, pilot testing of the materials should occur prior to field trials so that potential benefits and adverse impacts can be recognised and avoided. This experiment therefore aims to provide information and guidance on the likely impacts of organic materials to the mine tailings when conducting field trials on the study sites.

3.4.2 Overall Aims

The overall aim of the experiment is to assess the impact that locally sourced organic amendments have on the chemical, physical and biological functioning of mine tailings from Tyndrum and Le Bleymard. The experiment evaluates different organic material in the role of reducing PTE bioavailability, improving the nutrient status of the tailings and enhancing the microbial functioning within the tailing system.

Objectives

1. Design a novel experiment investigating the holistic effects of adding organic materials to the Tyndrum and Le Bleymard mine tailings.
2. Identify and explore the effects of different, locally relevant organic amendments when they are added to the tailings.
3. Identify statistical and methodological approaches that can critically evaluate these effects.
4. Consider the implications of using these materials on the abandoned mines during field trials.

3.4.3 Research Questions and Hypotheses

Research Questions

1. What organic amendments are available locally to each site? Are they suitable for incorporation into the mine tailings?
2. Following organic amendments what are the impacts on:
 - a) PTE fractionation?
 - b) Soil fertility properties (assessed as N, P, K concentrations, pH, microbial biomass, CO₂ evolution, moisture content etc.)?
 - c) Potential leachate or runoff risks?
3. Which organic amendment appears to be the most appropriate (including transportation, availability and cost considerations)?

Hypotheses

Hypothesis 1

H₀: The addition of organic amendments does not significantly alter the fractionation and availability of PTEs in the mine tailings.

H₁: The addition of organic amendments does significantly alter the fractionation and availability of PTEs in the mine tailings.

Hypothesis 2

H₀: The addition of organic amendments does not significantly improve the fertility, nutrient cycling and function of the mine tailings as a supporting medium for plants and soil biota.

H₁: The addition of organic amendments does significantly improve the fertility, nutrient cycling and function of the mine tailings as a supporting medium for plants and soil biota.

Hypothesis 3

H₀: The addition of organic amendments does not significantly promote the risk of PTE leachate and associated runoff from the mine tailings.

H₁: The addition of organic amendments does significantly promote the risk of PTE leachate and associated runoff from the mine tailings.

Hypothesis 4

H₀: There are no significant differences in the outcomes of hypotheses 1-3 between different organic amendments

H₁: There are significant differences in the outcomes of hypotheses 1-3 between different organic amendments

3.4.5 Organic Materials

The organic materials and wastes listed below have been identified as possible candidates for the experiment based on local availability and published NPK and DOC contents. The investigation uses materials that are locally relevant and also novel ones that can increase scientific understanding.

Green Waste Compost

Compost derived from green wastes (e.g. garden and food waste) have been extensively reported to produce positive impacts to metalliferous mine tailings in terms of nutrient enhancement, structural improvements and PTE immobilisation (Tandy *et al.* 2009; Novo *et al.* 2013; Beesley *et al.* 2014b). Stirling Council engages in a green waste collection system, with green and cardboard domestic waste collected and composted for use in allotments and gardens (Stirling Council 2016). The nearest composting site to Tyndrum is in Callander, approximately 35 miles south of the mine site. The GWC complies with cattle compost (BSI PAS 100) and costs £7 per tonne (smaller collections are free to residents). Compost schemes are freely promoted in the Lozere region, with several sites around Le Bleymard (Lozère 2017). Green waste

compost quality in France has been criticised as having less stringent regulations (WRAP 2015) in relation to inert contamination (e.g. glass, plastic and stone) and variability of plant nutrients.

Sheep Manure

Sheep manure (SM) and SM extract has been previously used as a soil amendment for PTEs and as a general soil conditioner (Safari Sinigani and Khalilikhah 2010; Pérez-Esteban *et al.* 2012). Sheep manure in its unprocessed form has not been as well studied, however it has some evidence of reducing Pb, Zn and Cd availability in contaminated soils (Han *et al.* 2013). Additionally, SM could act as a source of soil nutrients, microbial inoculation and as a seed bank (Williams and Haynes 1995; Kuiters and Huiskes 2010). As SM is not aged in regard to GWC processes it is likely that degradation and the subsequent chemical processes will be accelerated, potentially leading to a faster bio-remedial effect in the mine tailings. Sheep manure can be sourced in Tyndrum from the nearby, SRUC owned Kirkton Farm, whereas the nearest available resource in Le Bleymard is approximately 50 km south west.

Cattle Manure

Cattle manure (CM) has been used extensively for the treatment of PTE, with most studies reporting that the input of organic and inorganic (e.g. PO_4^{3-} , SO_4^{2-}) constituents aid in the chemical immobilisation of PTE (Minkina *et al.* 2011). Similarly to SM based organic amendments, CM has been demonstrated to provide additional biological support in terms of increasing organic carbon content, soil structure and important soil nutrients as well as improving the sequestration of PTEs (Walker *et al.* 2004; Wu *et al.* 2013; Galende, Becerril, Gomez-Sagasti, *et al.* 2014). Some concerns have been raised with the potential for Cu mobilisation via high inputs of DOC associated with CM and the possible increases in Cd and Zn due to bio-concentration in food chains resulting from drinking water, consumption of grass from pastures fertilised with Cd contaminated P sources or hoof disinfectant washes when spreading CM on soils (Benke *et al.* 2008; Wuana and Okieimen 2011). Due to the intrinsically high concentrations of PTE in the Tyndrum mine tailings small inputs of PTE via the

incorporation of CM are not expected to be significant. Cattle manure is readily available at both study sites from neighbouring farms.

Spent Coffee Grounds

Spent coffee grounds (SCG) have been identified as a potentially useful organic amendment, primarily for the high concentration of available OM compounds and as a potential source of N, P and K (Soto and Munoz 2002; Liu and Price 2011; Caetano *et al.* 2014). However, several studies have demonstrated that SCG can have detrimental impacts on soil health, inhibiting N mineralisation, suppressing seed germination and producing toxicological impacts to soil microbial communities (Cruz *et al.* 2012; Yamane *et al.* 2014; Cruz and Marques dos Santos Cordovil 2015; Aguiar *et al.* 2016; Hardgrove and Livesley 2016). A handful of studies have explored the use of SCG in their potential as PTE immobilisation materials, primarily due to the range of labile organic functional groups that can act as binding sites for PTEs and reduce their bioavailability in soil and water environments. Spent coffee grounds have been reported to be effective in removing Pb and Cd from water columns (Tokimoto *et al.* 2005; Azouaou *et al.* 2010) and use of SCG have been proposed for the removal of aqueous Cr(VI) via adsorption reactions. One study by Kim *et al.* (2014) demonstrated that concentrations of Cd, Cu, Pb and Zn were reduced in contaminated soils by the direct addition of SCG, proposing that S = O functional groups and organic ligands were reducing the bioavailable concentrations of these elements in the soil. The study however noted that despite the improvements in available PTE concentrations detrimental impacts on plant development occurred. Spent coffee grounds would be available at both sites from local villages.

3.4.6 Sample Collection

Organic amendments

Fresh CM and SM was collected from a field at SRUC's Boghall Farm estate. Approximately 5 kg of each manure was collected in individual clean plastic buckets, mixed thoroughly and stored for three days at 4°C until use. Green waste compost was obtained from SRUC's horticultural composting heaps which is

mainly comprised of decaying plant material and a small proportion of soil and other inert aggregates. Spent coffee grounds were acquired from several local coffee outlets in the City of Edinburgh and stored for two days at 4°C until use. Green waste compost and SCG were passed through a <2 mm sieve to remove any unwanted material such as stones and teabags. The collected materials were used as amendments for both study sites, as deviations in material composition are unlikely.

Tailing Sampling

Tailing material from both mines were sampled using ArcGIS to devise random sampling points within the defined site area (Figure 3-5 & Figure 3-6). From this, twenty random samples of approximately 1 kg were collected following the same procedures as section 3.2.2. These were bulked and passed through a <2 mm sieve. Tailing samples from Le Bleymard and Tyndrum were collected in April 2016 and July 2016 respectively.

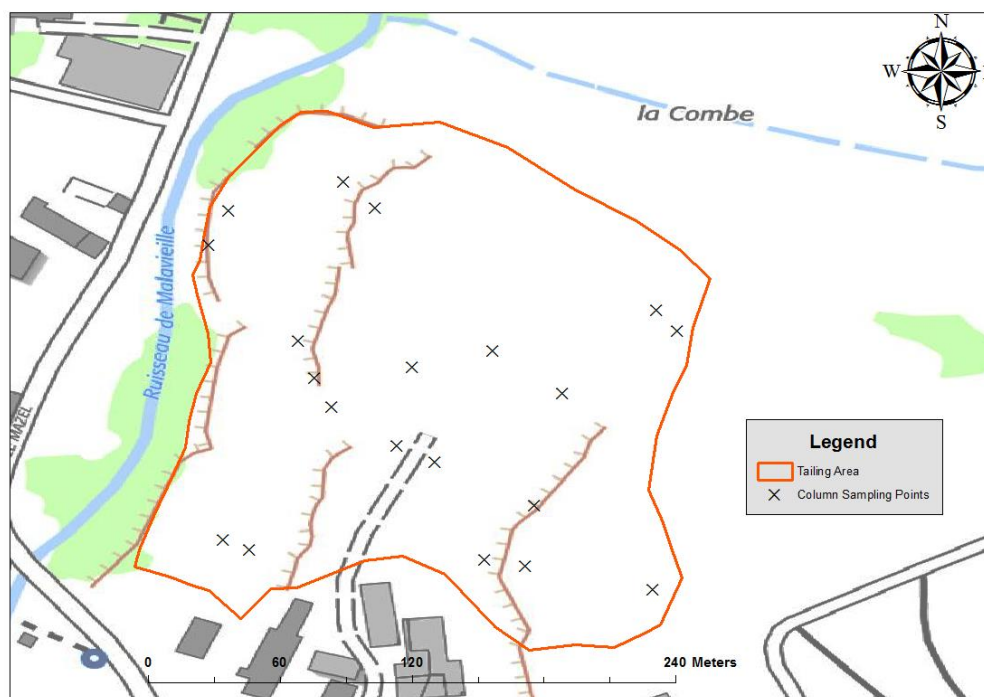


Figure 3-5. Random sampling points of collected tailing material for the organic amendment study from the Pb/Zn mine at Le Bleymard.

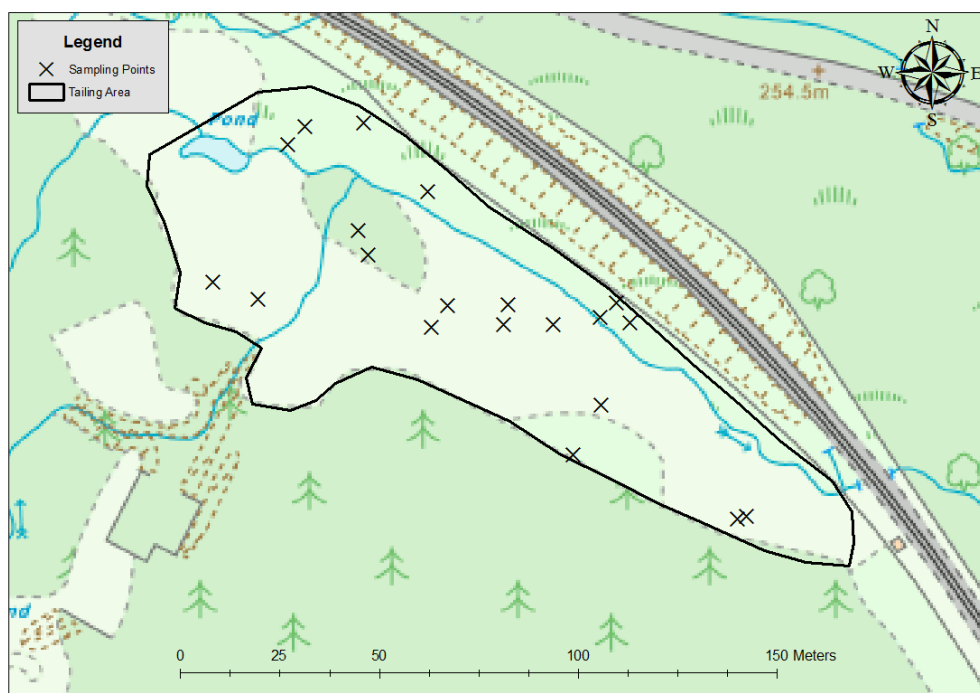


Figure 3-6. Random sampling points of collected tailing material for the organic amendment study from the Pb/Zn mine at Le Bleymard.

3.4.7 Column Design

Plastic PVC columns (dia. 55mm, height 300mm) were used to enable gas and leachate collection from the amended tailings. Caps containing polycarbonate luer stopcocks (Cole – Parmer), and bases with acid washed glass wool ($\leq 0.001\%$ PTEs (as Pb), Sigma-Aldrich) and glass beads (Sigma-Aldrich) were installed in each column to facilitate the collection of gas and leachate (Figure 3-7). Bases and lids were produced by using clean, 55 mm dia. petri dishes and drilling a small hole in the centre. All plastic-ware components were washed in 5% Decon90® for 48 hours followed by rinsing with excess de-ionised water.

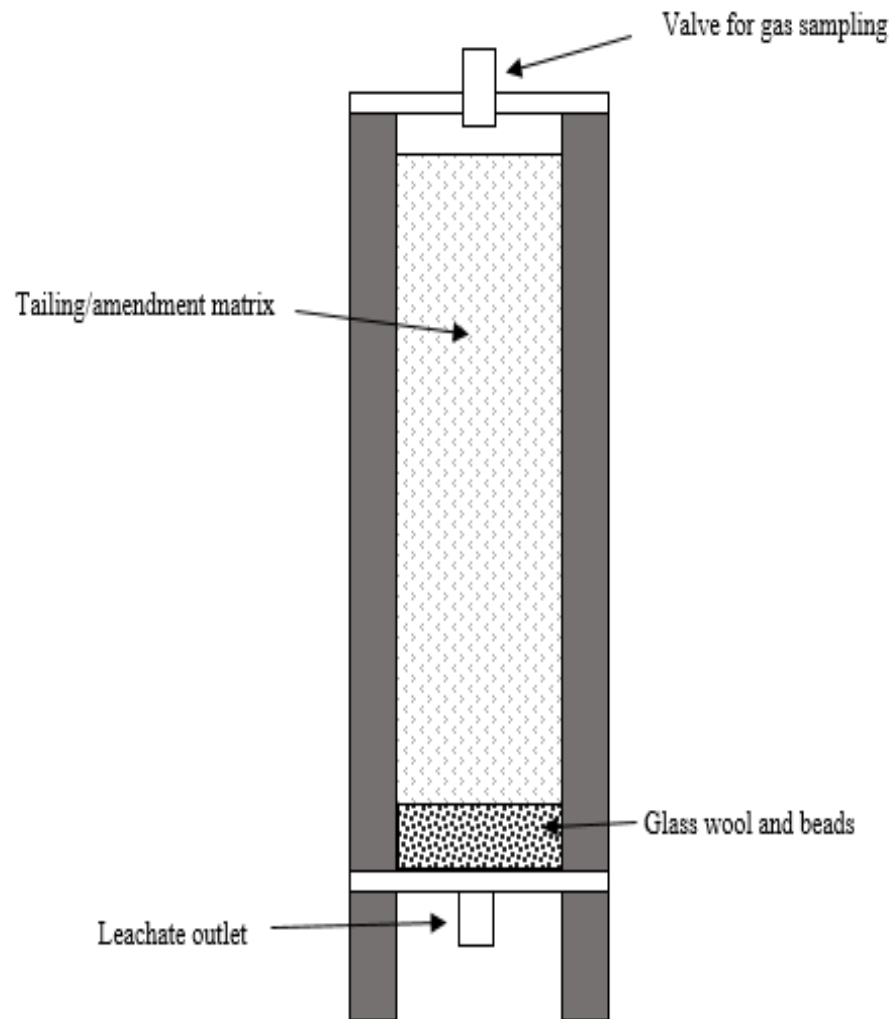


Figure 3-7. Schematic of column leaching system used in Chapter 6.

3.4.8 Preparation, Sample Arrangement and Incubation Environment

Homogenised mine tailings were mixed with the relevant organic material at an application rate equivalent to 150 (8%, low) and 300 (16% high) t ha⁻¹. These relatively high application rates which compared to conventional applications of animal and biosolid wastes (such as sewage sludge (40 t ha⁻¹) and poultry manure (8.5 t ha⁻¹) (EC 2001; Roig *et al.* 2012)) were chosen due to the poor structure and supportive nature for plant growth. Mixing was completed in a clean, plastic lined cement mixer (Belle, 90L capacity) at 24 rpm for 30 minutes.

Approximately 0.5 kg of the mine tailing/organic mix was carefully packed in the relevant column and moistened to 60% of its water holding capacity (WHC) using de-ionised water. Water holding capacity was determined by taking a sub-sample of each tailing/organic mix and proceeding following section 3.7.5, and each column was monitored and adjusted gravimetrically for water loss based on the initial moisture content.

The columns were incubated on a laboratory bench top at 15°C for four weeks with a 16-hour daylight regime. During incubation the columns were monitored for moisture content gravimetrically and wetted to maintain 60% WHC every two to three days as required. Samples were arranged in a blocking structure (generated using Minitab v. 16 software) and raised wooden tables with drilled holes were used to allow leachate collection (Figure 3-8). Four blocks containing 9 samples (1 repetition of each treatment plus 1 control) were used.



Figure 3-8. Column block set up for the organic amendment investigation.

3.4.9 Sampling Strategy

Column Gas Sampling

Column headspace gas sampling was conducted by collecting ambient air prior to sealing the columns using a polystyrene lid and polytetrafluoroethylene gas

tape, allowing gas in a 13 cm headspace height to accumulate over 60 minutes for carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) determination (Oertel *et al.* 2016). Gas was collected via a 50 ml syringe and injected into a previously evacuated glass vial until analysis. Vials were over-pressured to maintain sample preservation and analysed within one week.

Column Leachate Collection

Leachate samples were collected weekly by slowly bringing the WHC of the tailings to 120% with de-ionised water to induce leachate removal from the tailing column. The collected leachate was subsequently filtered via a Whatman grade 2 paper (< 8µm), then via a 0.45 µm PTFE syringe filter (Merck Millipore, Germany) and immediately analysed for DOC, pH and NO₂⁻-N+NO₃⁻-N/NH₄⁺-N. A separate aliquot was acidified with 100 µl of conc. HNO₃ (Aristar trace analysis grade, VWR), assessed for PTE and other elemental concentrations within 2 days of collection and the remaining samples frozen at -20°C.

Column Solid Material

Upon conclusion of the experiment the solid material from each column was thoroughly mixed and refrigerated at 4°C in separate, labelled polyethylene bags.

Statistical Analysis

Simple mass balance equations using the total concentration of PTE in the tailing – organic material matrix and the discharges of PTE from the tailing leachate were used to calculate recoveries.

The statistical package Origin 2016® was used in the production of figures, one-way and repeated analysis of variance (ANOVA), Pearson's correlation and cluster analysis. An Anderson –Darling Test was used to verify normality, and data were log transformed where necessary. Tukey's post hoc test was applied to identify differences between each treatment group, with a significance level of $P < 0.05$ used for all tests.

3.5 Field Trials

3.5.1 Introduction and Overview

Based on results from sections 3.3 and 3.4 a seven-month field trial was undertaken to assess the continuing changes of PTE fractionation and mine tailing development after organic amendments and vegetation were applied. The field trials followed a similar methodology to the column experiment, with analysis focusing on overall soil parameters and the changes in PTE fractionation and bioavailability.

Plastic rings, referred to as soil gas chambers, were used to delineate trial plots, with organic amendments mixed into the tailings inside the ring walls. At the Tyndrum Pb/Zn mine, two organic amendments, spent coffee grounds (SCG) and GWC were applied at a rate of 200 t ha⁻¹ with six replicates and sown with a mixture of seeds of *Agrostis capillaris* and *Festuca ovina*, which were identified from the results of Chapter 6 and 5 respectively. At the site at Le Bleymard GWC and CM was applied at a rate of 200 t ha⁻¹ alongside *A. capillaris* and *F. ovina*, (see Chapters 5 and 6). Furthermore, only 5 replicates were used due to limitations in access.

The trials were monitored for approximately seven months at each site, with monthly sampling completed to critically evaluate changes over time at Tyndrum and a 3-month sampling regime at Le Bleymard, consisting of two monitoring stages and a final destructive sampling stage.

3.5.2 Research Questions

PTE Fractionation/Speciation

1. What are the changes (if any) in the PTE fractionation in the treatments compared to the control?
2. Are there speciation changes in the soil (specifically for Pb and Zn) after remedial action occurs?
3. Overall is there a change in 'bioavailable' PTE concentrations?

Biological Changes

1. Has soil nutrient availability and the potential pool of essential and micro elements/nutrients increased?
2. Are their changes in microbial functioning and development?
3. Is there an improvement in vegetative development and support for plant species?
4. Are changes in ecotoxicological risks apparent from the remedial strategies i.e. translocation of PTE into aerial compartments of plants?

3.5.3 Organic Amendments and Trial Design

At each site, the trial design consisted of two organic amendments; one application rate of 200 t ha⁻¹ and six (Tyndrum) or five (Le Bleymard) replications, with vegetation. The plant species used was a 1:1 seeding ratio of *A.capillaris* and *F.ovina*, based on vegetative success in Chapter 5. In addition to the treatments 5 or 6 un-amended replicates were included as a control, with the tailings disturbed in a similar manner to the amended replicates.

The organic amendments were GWC and SCG (Tyndrum) and GWC and CM (Le Bleymard). These choices were established from results of Chapter 6. Both amendments were sourced locally where possible.

3.5.4 Detailed Description of Plots

The trial plots utilised gas chambers to simultaneously provide infrastructure for measuring soil gas fluxes and to provide a contained area for the trial plots, with an amendment depth of 30 cm. The chambers were bottomless, with a diameter of 38.5 cm a height of 30 cm and a surface area of 0.12 m².

At both sites, the chambers were imbedded approximately 15 cm into the mine tailings, ensuring that appropriate headspace was available for gas flux measurements. The organic amendment was mixed thoroughly into the tailing substrate using a spade at an application rate of 200 t ha⁻¹ fresh mass, ensuring that a homogenous profile was obtained. To each plot a 1:1 seeding ratio, totalling 150g, of *A.capillaris* and *F.ovina* were added. A distance of approximately 30 cm was left between each replicate and the location of each replication point was fully randomised within the trial area.

In Tyndrum three Rhizon® samplers (model *Rhizon Flex* , 19.21.26) were introduced at a depth of 5, 10 and 20 cm in each replicate treatment and left for at least 7 days before analysis to allow the tailing matrix to settle (Figure 3-9). Rhizon samplers were left in situ until the conclusion of the experiment and replaced when necessary (e.g. in the case of damage or blockage). The use of Rhizon® samplers at Le Bleymard was not possible due to the aridity of the tailings and difficulty in collection and sample preservation.

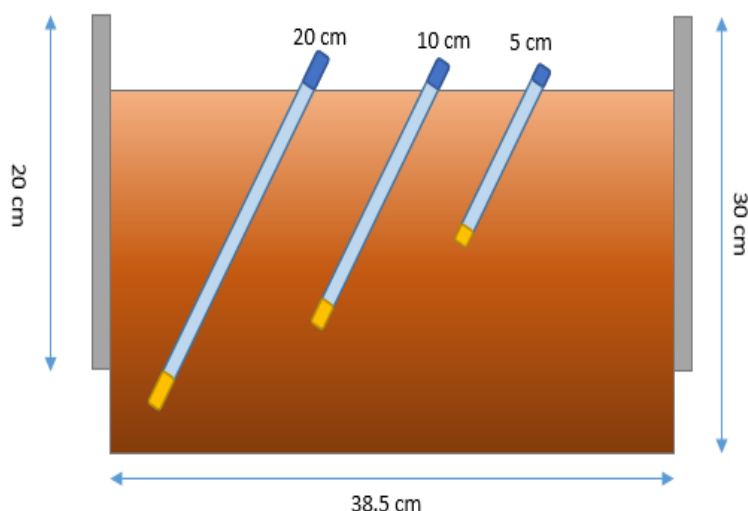


Figure 3-9. Cross section of the field trial plot design at Tyndrum.

The plots were located as shown in Figure 3-10 and Figure 3-11. A Garmin eTrek (acc. ± 1.1 m) was used to accurately locate the trial sites and each point was clearly marked. Field trials at Tyndrum and Le Bleymard were established on the 2 March and 6 of April 2017 respectively. At each plot location three replicate chambers were installed, one un-amended control, one GWC and the site-specific amendment for each respective site.

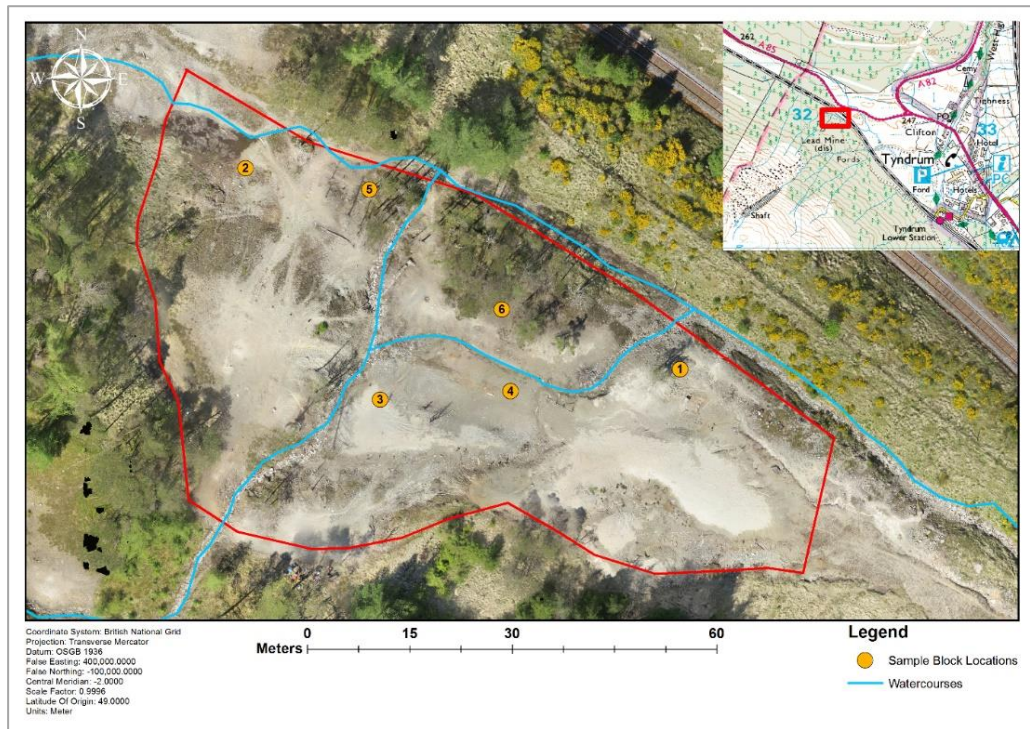


Figure 3-10. Plot locations at the Tyndrum mine

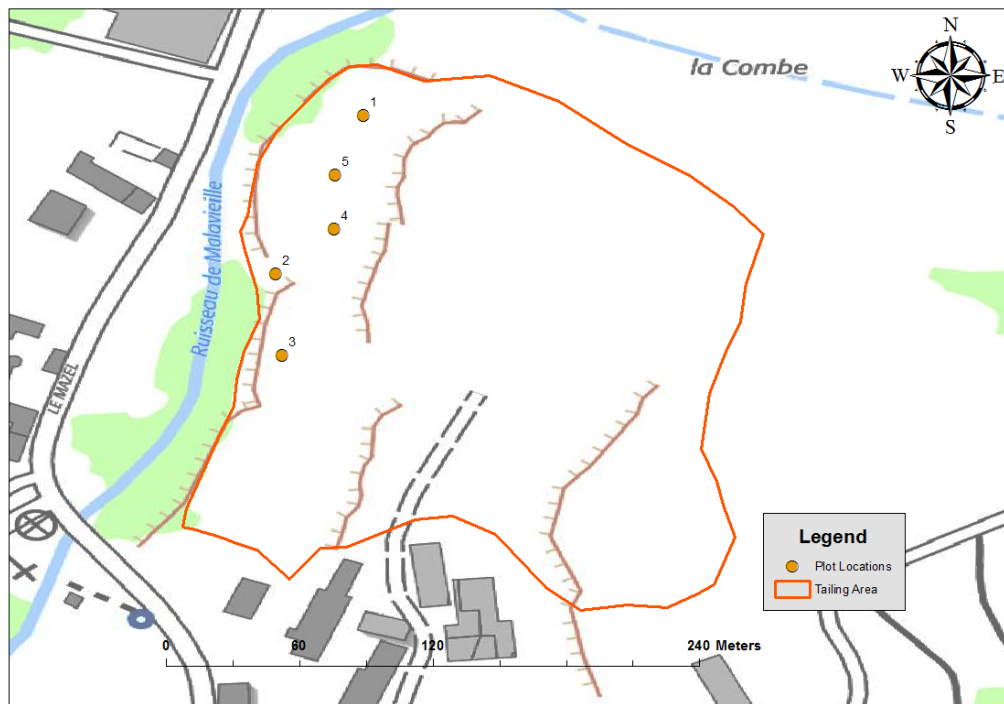


Figure 3-11. Plot locations at the Le Bleymard mine

3.5.5 Sampling Regime

Tyndrum

Monthly sampling (approximately the 15th of each month) involved gas sampling, vegetation measurement and the removal of pore water from the Rhizon samplers. Sampling was avoided during very heavy rainfall to minimise health and safety hazards and prevent issues surrounding gas sampling under waterlogged conditions.

Le Bleymard

Sampling which occurred in June, August and October 2017 involved the measurement of vegetation height and cover only. Parallel to the Tyndrum site, sampling visits avoided adverse weather where possible.

Pore Water Sampling

Pore water was removed by utilising pre-evacuated, sterile vacuum tubes and a syringe to draw water from the soil into the vessel, minimising soil disturbance and reducing issues created by redox change. Pore water samples were stored in a cool box and stored at 4°C until analysis. Filtration of the pore water was unnecessary as the Rhizon samplers have a pore size of 0.12 – 0.18 µm.

Gas sampling

Gas samples were taken at two intervals: zero and 60 minutes (T_0 and T_{60}) to calculate daily flux. At T_0 ambient air was taken from the chamber, the chambers were then sealed using an aluminium plate with a built-in valve for sampling and two replicate samples were drawn at T_{60} . Samples were kept in over-pressurised, pre-evacuated gas vials for a maximum of three days. Upon completion the aluminium plates were removed to allow recirculation and rainfall infiltration.

Vegetation Monitoring

Vegetation was monitored by measuring height as described by (Perez-Harguindeguy *et al.* 2016) and the percentage of total cover using a 30 cm² quadrat in the soil chamber. Additionally, general notes on discolouration, signs of chlorosis or disease etc. were recorded.

Final Sampling

Destructive sampling occurred upon the conclusion of the investigation, on the 28th of September 2017 and 23rd of October 2017 for Tyndrum and Le Bleymard respectively. A tailing core (Ø 110mm x 20 cm) was taken from each trial plot and placed in a clean labelled polyethylene bag.

Tailing samples were analysed for BCR extractable elements, total elemental concentrations, pH, NO₃⁻/NH₃, EC, TC, TN, cold water extractable carbon (CWECC) hot water extractable carbon (HWECC) and loss on ignition (LOI). Vegetation samples were assessed for root and shoot biomass and total elemental concentrations in root and shoot materials.

3.5.7 Statistical Analysis

Several statistical approaches were used to assess the effectiveness of the experimental treatments. Analysis of variance (ANOVA, MANOVA, Analysis of Means) and multi-variate methods (e.g. principal component analysis, multiple linear regression) were considered the most appropriate tools for this investigation to allow for several important parameters to be considered at once. Statistical measurements were completed using Minitab v. 18, with a significance level of $P < 0.05$ used for all tests.

3.6 Sample Preparation and Analysis

3.6.1 Glassware and Plasticware Cleaning

Elemental Analysis

Prior to use in elemental assays, glassware was immersed in 5 mol L⁻¹ Analar® nitric acid and boiled for four hours on a hotplate. The glassware was subsequently rinsed with de-ionised water and then submerged in de-ionised water and boiled for a further four hours. Upon cooling, the glassware was rinsed again with de-ionised water and placed in a clean drying cupboard. Plasticware was washed by immersing in 5% v/v Analar® nitric acid for 24 hours, rinsed with de-ionised water and placed in a clean drying cupboard.

Microwave vessels (section 3.7.12) were cleaned immediately after each analytical run by transferring 10 ml of concentrated nitric acid (Analar®) into

the relevant vessel and processed using a cleaning microwave protocol (Table 3-4). The cleaning acid was decanted and all parts of the vessel and thoroughly rinsed with de-ionised water before placing the vessels into a clean drying cupboard.

Table 3-4. Cleaning protocol for the Mars6 digestion vessels.

Mars6 Parameter	Value
Name	'Clean1'
Power	1200
Ramp Time	20 minutes
Hold Time	10 minutes
Initial Temperature	20°C
Final Temperature	180°C
Initial Pressure	0 psi
Final Pressure	150 psi

NO₃⁻/NH₃ MBC

Prior to use, plasticware was soaked for 24 hours in a 5% v/v solution of Decon-90®, thoroughly rinsed with de-ionised water and allowed to dry.

3.6.2 Preparation of Solid Phase Samples for Elemental Analysis, TC/TN and XRD

Collected samples were air dried until a constant moisture content was obtained (approx. 15 days), passed through a 2 mm stainless steel sieve and milled to a fine powder using a RETSCH Mixer Mill 400. Milling vessels were cleaned thoroughly with de-ionised water between samples. The milled samples were stored in 30 ml polyethylene scintillation vials at room temperature until analysis. For TC and TN, a sub-sample of the milled matrix was removed, placed in an oven at 107°C (± 2) for 24 hours and stored in a 5 ml glass vial in a desiccator until analysis. For XRD samples, oven drying as per TC/TN was replicated with an additional combustion treatment in porcelain crucibles in a muffle furnace at 450°C for four hours, and the samples stored in 5 ml glass vials in a desiccator until analysis.

3.6.3 Preparation of Solid Phase Samples for NO₃⁻/NH₃ MBC, pH, EC and CWEC/HWEC

The collected samples were sieved through a 2 mm stainless steel mesh and kept in clean polyethylene bags in the dark at 4°C until further use. For NO₃⁻/NH₃ and MBC, samples were stored for a maximum of seven days before analysis. For pH, EC and CWEC/HWEC procedures samples were analysed within two weeks.

3.6.4 Preparation of Plant Material for Biomass, Elemental Analysis, TC and TN Analysis

Whole plants, including the root system, were gently removed from the tailings and were held over a clean polyethylene bag and shaken vigorously for 10 seconds to remove the bulk material, followed by further shaking in a separate polyethylene bag until the majority of the adhered soil was removed, termed rhizosphere material. The plants were subsequently separated into aerial and subterranean sections using a pair of polytetrafluoroethylene coated scissors and washed three times in de-ionised water or until the washing solution did not contain any noticeable soil particles (Smith *et al.* 2009).

After washing, the plants were air dried on paper towels to remove excess moisture and placed in previously weighed paper bags, the mass of the plant noted and then transferred to a drying oven at 70°C for 24 hours. This drying process also determined the dry weight plant biomass (section 3.7.1). The dried material was ground to a fine powder using a RETSCH Mixer Mill 400, with milling vessels cleaned thoroughly with de-ionised water between samples. The processed material was stored in a desiccator at room temperature until analysis.

3.6.5 Storage and Preparation of Leachate and Water Samples

Collected leachate and water samples were immediately stored in the dark in sterile polyethylene (Falcon™) tubes at <4°C after prior filtration through a 0.22µm hydrophilic membrane (Millex). Samples designated for elemental analysis were acidified with 100 µl of conc. Aristar grade HNO₃ to achieve a pH of around 2 and analysed within two weeks. For untreated samples analyses were completed within two days of collection.

3.7 Analytical Techniques

3.7.1 Measurement of Plant Dry Weight Biomass

From the drying process in section 3.6.4 plant dry weight biomass was calculated using Equation 3.

$$\text{Dry Weight Biomass} = \text{Fresh plant mass (g)} - \text{Oven dried (70°C) plant mass (g)}$$

Equation 3. Calculation for the determination of plant dry biomass.

3.7.2 Measurement of pH in Aqueous and Solid Samples

Aqueous samples

Aqueous samples were directly assessed for pH using a probe (Jenway 3540 with a Tuff-Tip® electrode (Cole -Parmer)) calibrated immediately before use using Traceable™ pH Buffer Standards 4.005 and 7.000. Calibration checks were completed every 10 samples and at the end of each run to ensure consistence in measurements.

Solid samples

One gram of previously prepared sample (section 3.6.3) was added to 5 ml of de-ionised water and shaken end over end at 30 rpm for 30 minutes in a 50 ml centrifuge tube, allowed to settle for 10 minutes and the slurry analysed directly using the same procedure as described above.

3.7.3 Measurement of Electrical Conductivity in Aqueous and Solid Samples

Aqueous samples

Aqueous samples were directly assessed for EC using a probe (Model HI2003-01, Hanna Instruments) calibrated immediately before use using 0.01, and 0.1 mol L⁻¹ solutions of KCl (>99.0%, Sigma-Aldrich) and de-ionised water at a temperature of 20°C to give standards of 1413, 12,880 µS/cm respectively. Calibration checks were completed every 10 samples and at the end of each run to ensure consistence in measurements.

Solid samples

One gram of previously prepared sample (section 3.6.3) was added to 5 ml of de-ionised water and shaken end over end at 30 rpm for 30 minutes in a 50 ml

centrifuge tube, allowed to settle for 2 - 3 minutes centrifuged at $5,000 \times g^{-1}$ and the decanted supernatant analysed directly using the same procedure as described above.

3.7.4 Organic Matter Percent via Loss on Ignition

Loss on ignition is a simple method to calculate the organic component of a sample by volatilising organic material at a high temperature and measuring the mass lost in the sample. Approximately 0.25 g of prepared sample (section 3.6.2) were accurately weighed into clean, previously weighed beakers and placed into an oven at $107^{\circ}\text{C} (\pm 2)$ for 24 hours, reweighed after cooling and ashed in a muffle furnace at 450°C for four hours. The residue was weighed once more and the loss of organic matter calculated (Equation 4).

$$\text{Organic Matter \%} = \frac{(\text{pre} - \text{ignition mass}) - (\text{post} - \text{ignition mass})}{\text{pre} - \text{ignition mass}} \times 100$$

Equation 4. Calculation for determining loss on ignition.

3.7.5 Measurement of Water Holding Capacity

The maximum WHC of samples was determined by weighing approximately 10g of sample into pre-weighed stainless-steel rings (diam. 50mm) with a 0.7mm muslin mesh base. The samples were placed in a tray with water added to the rim of the rings and allowed to saturate overnight. The samples were removed from the water and weighed after dripping was no longer observed (approximately 1 hour). The samples were then placed in an oven at $107^{\circ}\text{C} (\pm 2)$ for 24 hours and the WHC determined by calculating the mass of water lost.

3.7.6 Measurement of $\text{NO}_3^-/\text{NO}_2^-$ and NH_3 in Aqueous and Solid Samples

Method Principals

Available N in the form of $\text{NO}_3^-/\text{NO}_2^-$ and NH_3 was directly analysed following an automated Cd-reduction method and a modified Berthelot reaction on a Skalar San⁺⁺ Wet Chemistry Analyser (Skalar). For $\text{NO}_3^-/\text{NO}_2^-$, the sample is first passed through a Cu/Cd column to reduce nitrate to nitrite and the emerging nitrite is diazotized with sulphanilamide and complexed with N-(1-naphthyl)-ethylenediamine dihydrochloride to generate a pink coloured solution which is

measured at 540 nm. For NH_3 , ammonia in the sample is chlorinated via hypochlorite to produce monochloroamine and subsequently reacted with salicylate to form 5 - aminosalicylate. Oxidative reactions then promote the formation of indophenol blue which is intensified by the addition of sodium nitroprusside and directly proportional to the concentration of ammonia in the sample. The coloured solution is subsequently measured at 660 nm.

Calibration standards for $\text{NO}_3^-/\text{NO}_2^-$ and NH_3 (Table 3-5) were created using sodium nitrate (99.995%, Sigma - Aldrich) and ammonium chloride (99.998%, Sigma -Aldrich) in deionised water and 2 mol L^{-1} KCL (>99.0%, Sigma-Aldrich) as required.

Table 3-5. Calibration standards used in the analysis of $\text{NO}_3^-/\text{NO}_2^-$ and NH_3

Calibration Standard	NO_3^- (mg L^{-1})	NH_3 (mg L^{-1})
Standard 1	5.00	10.00
Standard 2	4.00	8.00
Standard 3	3.00	6.00
Standard 4	2.00	4.00
Standard 5	1.00	2.00
Standard 6	Calibration Blank	Calibration Blank

Quality Control Measures

During operation drift (Standard 2) and blank checks were completed every ten samples to assess instrument functioning and a double set of standards were performed at the beginning and end of each run. All samples were analysed in duplicate to ensure similarity of outputs within and between samples.

Aqueous samples

Prepared (section 3.6.5) aqueous samples were measured directly using de-ionised water calibrations. Samples were diluted where required to fall within the calibration range as stated in Table 3-5.

Solid Samples

Ten grams of previously prepared (section 3.6.3) samples were placed in clean 100 ml polyethylene jars with 20 ml of 2 mol L^{-1} KCL and shaken at 200 rpm for one hour on an orbital shaker (Thermoline Scientific). Samples were then left

to settle for 10 minutes and a 10 ml aliquot was transferred to a 15 ml centrifuge tube and centrifuged at $15,000\text{ g}^{-1}$ for 15 minutes. The supernatant was decanted into disposable polyethylene tubes and analysed on the same day using matrix matched standards. Concentrations of solid phase $\text{NO}_3^-/\text{NO}_2^-$ and NH_3 were calculated as following Equation 5.

$$\text{Concentration (mg/kg)} = \frac{\text{Conc. (mg/L)} \times \text{Extractant Volume (L)} \times \text{Dilution Factor}}{\text{Mass (kg)}}$$

Equation 5 Calculation for the determination of $\text{NO}_3^-/\text{NO}_2^-$ and NH_3

3.7.7 Measurement of DOC in Aqueous Samples

Method Principals

Dissolved organic carbon in aqueous solutions is measured by converting solution-held organic matter into CO_2 via acidification with 25% v/v orthophosphoric acid and subsequent purging of bicarbonates and carbonates from solution using N_2 to remove CO_2 . From there, residual organic carbon is oxidised to CO_2 via UV excitation and reaction with persulfate and measured by non-dispersive infra-red spectrometry.

In this investigation, a Rosemount Dohrmann DC-80 Total Organic Carbon Analyser was used to assess solutions of previously prepared samples. For each measurement 100 μl of 25% v/v orthophosphoric acid was added to 5 ml of sample and purged with N_2 for 5 minutes before a 200 μl aliquot was manually injected into the instrument and measured as DOC (mg L^{-1}).

A single calibration point was produced as recommended by the manufacturer, comprising of two 400 mg L^{-1} standards using potassium hydrogen phthalate (99.95%, Sigma-Aldrich) and matrix matched to the analyte solution.

Quality Control

Due to the single calibration point, double calibration standards were produced to measure accuracy of outputs and a reference material (76067 Sigma-Aldrich TOC – 1000 mg/L^{-1} ($\pm 10\text{ mg L}^{-1}$)) diluted to 1, 50 and 100 mg L^{-1} and matrix matched was used to verify results. Calibration checks, internal reagent blanks,

external solution blanks and reference material checks were performed every 15 samples to assess the integrity of the instrument and detect issues with instrumental drift. All samples were analysed in duplicate to ensure similarity of outputs within and between samples.

3.7.8 Measurement of Microbial Biomass Carbon

Method Principals

Microbial biomass carbon quantification is widely considered to represent the mass of soil biota less than 5 – 10 μm^3 (Joergensen 1995) and is of interest to soil scientists due to its use as an indicator of ecological functioning and the substantial importance of microbial communities in terms of nutrient cycling, soil formation and support for higher organisms e.g. (Kaschuk *et al.* 2010; Habashi 2016). Several methods for measuring microbial biomass exist, such as staining of microbial cells, assessment of soil respiration and the release of carbon after fumigation and lysis of cells (Anderson and Domsch 1978; Joergensen and Brookes 1990; Beck *et al.* 1997).

The fumigation method based on Beck *et al.*, (1997) was applied in this investigation, where matched, un-fumigated and chloroform exposed samples are compared to assess the release of C from lysed cells. For un-fumigated samples, a 15 g sub sample (section 3.6.3) is weighed into a clean 100 ml polyethylene jar with 80 ml of 0.5 mol L⁻¹ K₂SO₄ (> 99.0%, Sigma-Aldrich) and shaken for 30 minutes at 200 rpm and allowed to settle for 10 minutes. Approximately 25 ml was decanted into a 50 ml centrifuge tube and centrifuged at 15,000 x g⁻¹ for 15 minutes before decanting the supernatant into a clean polyethylene scintillation vial. The sample was immediately analysed following section 3.7.7. For fumigated samples, 15 g sub-samples were placed in clean, shallow 50 ml glass dishes and placed in a vacuum oven at 25°C with chloroform (>99%, stabilised with amylenes, Sigma-Aldrich) and evacuated to approximately 25 kPa⁻¹ until the chloroform was seen to visibly boil. The samples were incubated for 24 hours under these conditions, after which the chloroform was purged from the samples and the fumigated matrix extracted following the same procedure as non-fumigated samples.

The extracted samples were analysed directly following the procedure in section (3.7.7). To calculate the microbial biomass carbon was used Equation 6.

$$MBC (mg/kg) = \frac{(Fumigated\ sample_{(mg/L)} - Unfumigated\ sample_{(mg/L)}) \times Extractant\ Volume\ (L)}{Mass\ of\ sample\ (kg)}$$

Equation 6. Calculation for the determination of MBC.

3.7.9 Cold and Hot Water Extractable Carbon

Method Principals

Cold and hot water extractable C (CWECH/HWEC) is often applied to agricultural soils to gain an indication of the immediately available and potentially available pools of microbial accessible carbon in a soil system, as well as microbial biomass and soil aggregation (Ghani *et al.* 2003). Furthermore, the extraction process concurrently elutes other important soil nutrients such as N compounds, phosphates and sulphates, allowing a holistic estimation of soil quality as it is suggested that a decrease in HWEC will also reflect a reduction in these soil parameters (Ghani *et al.* 2003). The process is based on a sequential extraction procedure where the same sample is extracted first with cold water and then by hot water to gauge the concentration of labile C in both fractions.

Extraction Procedure

Three grams of previously prepared sample (section 3.6.3) were accurately weighed into a 50 ml centrifuge tube with 30 ml de-ionised water and shaken at 30 rpm, end over end for 30 minutes at 20°C. After allowing to settle for 5 minutes the samples were centrifuged for 20 minutes at 5000 x g⁻¹, the supernatant filtered through a 0.45µm hydrophilic membrane (Millex) and stored in the dark at 4°C in a clean polyethylene scintillation vial. This extraction is termed CWECH. For HWEC, a further 30 ml of de-ionised water was added to the residue, placed on a vortex for 10 seconds and incubated in a water bath (Fisher, Model 202) at 80°C for 16 hours. Subsequently, the sample was agitated on a vortex for 10 seconds and extracted as per CWECH. Both CWECH and HWEC were analysed following section (3.7.7) on the same day as the HWEC procedure.

3.7.10 Total Organic Nitrogen and Total Organic Carbon

Method Principals

Total C and N content of a soil is a useful indicator for assessing the ability for a substrate to support vegetative and microbial functioning and to provide information on the C:N ratio of the sample. This ratio is important in determining the potential of the tailing matrices to provide a balanced source of both elements for microbial growth, as a high presence of C can lead to microbial depletion of tailing N (USDA 2011), and a resulting nutrient deficit for plants. Therefore, understanding the C:N ratio of remediated tailing substrates is important for optimising vegetative growth.

The most common approach for simultaneously measuring TC and TN is based on the Dumas method where soil is combusted at high temperatures in the presence of oxygen to produce simple gases such as CO₂ and N₂ which are then separated via gas chromatography. In this investigation a Thermo Scientific Flash 2000 Elemental Analyser was used to calculate TN and TC of previously prepared samples (section 3.6.2). Introduced samples are exposed to a highly oxidative environment and the tin container aids in the triggering of a substantial exothermic reaction of temperatures up to 1,800°C. The combustion products are passed through a reaction column consisting of quartz wool, reduced Cu, Cr₂O₃ and silvered copulates which completes the oxidation process and reduces N species to N₂. The gases are subsequently passed through a 2 m steel chromatographic column and determined by a thermal conductivity detector. Concentrations of TN and TC are automatically calculated by the instrument based on the mass of the sample and displayed as a percentage.

Sample Preparation and Procedure

For each sample between 15 – 20 mg was accurately weighed into tin capsules (8 x 5 mm, Elemental Microanalysis) and placed in a clean 96 well microplate (Thermo Scientific) in a desiccator until analysis.

Samples were loaded into an auto sampling carousel and dropped into the instrument furnace as the previous sample reached completion.

Calibration and Quality Control

A two-point calibration curve was produced by accurately recording the mass of approximately 10 mg of aspartic acid (European Pharmacopoeia Reference Standard, Sigma-Aldrich) and a blank capsule. For quality assurance, a Thermo Scientific N/C Soil Standard and a blank capsule was included at least every 10 samples, with a final aspartic acid, soil standard and blank sample at the end of each analytical run.

3.7.11 Gas Chromatography of Carbon Dioxide, Nitrogen and Methane

Method Principals

Gas chromatography is based on the separation of compounds based on their residence time in a separation column. Gas chromatography uses two phases, the mobile phase which consists of a carrier gas such as helium (He) or argon (Ar), and a stationary phase. The stationary phase can either be in the form of a column packed with fine particles that may or may not be coated with a high boiling polymer (liquid phase), or a thin, (0.2 – 0.5 mm) capillary tube coated with the liquid phase. The separation process is centred on the interaction of the target compounds with the stationary phase, where intermolecular forces elicit different retention times in the column depending on the molecule in question, thus different compounds will be separated as a function of time and will enter the detection system at separate intervals, allowing the qualitative characterisation of a sample. Key factors in the separation process include vapour pressure, polarity differences in the compounds, stationary phase and the length and temperature of the column. The detection system can vary from mass spectroscopy methods, flame ionisation (FID) and electron capture detection (ECD) and thermal conductivity detection (TCD).

Gas Chromatography Analytical Procedure of Soil Gases

In this investigation collected gas samples were analysed directly using an Agilent 7890A GC System with a FID, TCD and ECD allowing the simultaneous collection of CH₄, CO₂, and N₂O respectively. Gas samples (sections 3.4.9 and 3.5.5) were loaded onto a CTC Combi Pal auto-sampling system (CTC

Analytics) after using a needle to release excess pressure from the vials. The samples were processed using the following parameters (Table 3-6).

Table 3-6. Gas chromatography parameters for the simultaneous measurement of CH₄, CO₂, and N₂O.

Agilent 7890A Parameter	Value	
Pressures	Front inlet (helium) - 220 kPa	
	Pneumatic control module B (PCM B) (helium) - 208 kPa	
	Air control set (air) - 300 kPa	
Gas Flows	Front inlet septum purge (helium) - 10.3 ml min ⁻¹	
	PCM B (helium) - 6.5 ml min ⁻¹	
	Column (helium) - 6.6 ml min ⁻¹	
	Front FID	(hydrogen) - 36.0 ml/min ⁻¹ (air) - 354 ml min ⁻¹
	Back ECD (nitrogen) 9.4 ml min ⁻¹	
	Aux TCD makeup (helium) – 5.2 ml min ⁻¹	
	Aux TCD reference (helium) – 24.9 ml min ⁻¹	
Temperature	Front inlet – 50 °C	
	Valve box – 100 °C	
	Front detector – 200 °C	
	Back detector – 250 °C	
Signals (20 Hz)	Front detector (FID) – 10.1 pA	
	Back detector (ECD) – 151 Hz	
	Aux detector (TCD) – 14.4 μV	

Gas Flux Calculations

Daily gas fluxes were calculated following Equation 7:

$$F = p \left[\frac{V}{A} \right] \frac{\Delta c}{\Delta t} \frac{273}{(T + 273)}$$

Equation 7. Calculation used for the determination of daily gas fluxes. Where: F = daily gas flux, P = gas density, V = volume of column, A = basal area of column, V/A = headspace height, $\Delta c/\Delta t$ = change of gas concentration over time (ppm/hour⁻¹), T = air temperature of column (Kelvin).

3.7.12 Microwave Digestion of Solid Matrices

Method Principals

The basic principal of microwave digestion is the chemical decomposition of a solid matrix in a sealed, pressurised container using heated, concentrated acids or other solvents via microwave radiation as an energy source. This approach to sample dissolution is favoured over conventional open vessel methods due to lower reagent volumes, increased decomposition products and greater reproducibility e.g. (Xing and Yeneman 1998; Sandroni and Smith 2002; Da Silva *et al.* 2014). Moreover, the approach tends to exhibit greater user safety and faster process speeds than traditional methods and so is has developed into a standardised way to prepare samples for elemental analysis.

In this research a Mars6 (CEM) microwave system was used to determine the total concentration of PTEs in solid samples. This system used 100 ml perfluoroalkoxy alkane vessels (HP 500 – Plus, CEM) with a fibreoptic temperature probe and an ESP Plus Pressure Sensor Kit to monitor pressure and temperature in a designated control vessel, which is then used as a reference for the neighbouring vessels in the analytical system. The procedure consisted of a ramping period to reach the target temperature followed by holding the sample at a given temperature and pressure for the specified time. Upon completion, the Mars6 would initiate a cooling programme to reduce the sample temperature to 70°C before the operator could remove samples from the instrument.

Procedure for Solid Tailing Samples

The digestion procedure followed a modified EPA 3052 method, where the modification included an additional sample preparation stage via loss on ignition (section 3.7.4) of previously prepared and accurately weighed 0.25 g samples (section 3.6.2). The incinerated sample was transferred quantitatively to a microwave vessel using 9 ml of concentrated nitric acid (Aristar®, 69% w/v, VWR International Ltd) and a further 1 ml of hydrofluoric acid (Aristar®, 48% w/v, VWR International Ltd) was added to promote the dissolution of silicate materials. The microwave vessels were capped and seated in the microwave carousel with the temperature and pressure sensors installed into the reference vessel. The vessels were then processed using parameters in Table 3-7.

Table 3-7. Operational parameters of the digestion of solid matrices following EPA 3052.

Mars6 Parameter	Value
Name	EPA 3052
Power	1200
Ramp Time	20 minutes
Hold Time	9 minutes
Initial Temperature	20°C
Final Temperature	180°C
Initial Pressure	0 psi
Final Pressure	350 psi

Upon digestion the samples were transferred quantitatively to 100 ml polytetrafluoroethylene beakers using small volumes of 2% v/v nitric acid (Aristar®, 69% w/v, VWR International Ltd) and reduced in volume on a hotplate (Stuart, CB-500) until 1-2 ml remained. The concentrate was quantitatively transferred to a 25 ml volumetric flask, washing the beaker with 2% v/v nitric acid and made up to the mark accurately with 2% v/v nitric acid. The solution was clarified by passing through a Whatmann 540 hardened, ashless filter paper (SigmaAldrich) and stored in a 30 ml polyethylene steralin tube (Thermo Scientific) in the dark at 4°C until analysis.

Procedure for Plant Samples

Plant samples were digested following a CEM Mars6 digestion method (CEM 2016). From section (3.6.4) 0.5 g of prepared plant sample was accurately weighed directly into the microwave vessel, saturated with 10 ml conc. nitric acid (Aristar®, 69% w/v, VWR International Ltd) and swirled gently. The vessel was left open to digest for 15 minutes before sealing, placing in the microwave unit as section (3.7.12) and processed under the following parameters in Table 3-8.

Table 3-8. Operational parameters of the digestion of plant material following a CEM digestion method.

Mars6Parameter	Value
Name	Plant Material
Power	1050
Ramp Time	20 minutes
Hold Time	15 minutes
Initial Temperature	20°C
Final Temperature	200°C
Initial Pressure	0 psi
Final Pressure	350 psi

The digestion process was completed following the remainder of section 3.7.12 with the substitution of polytetrafluoroethylene flasks for 100 ml borosilicate beakers as hydrofluoric acid was not present in the procedure.

Quality Control

For each microwave digestion batch two analytical blanks and two certified reference materials were included in each run to assess the efficiency and quality of the digestive process. Certified reference materials were matrix matched as close as possible to the environmental sample (e.g. plant material would be processed alongside a vegetative reference standard). References used are detailed in Table 3-9.

Table 3-9. Certified reference materials used in the PTE analysis of solid samples.

Reference Material	Matrix	Supplier
CRM 7002	Light sandy soil	ANALYTIKA Ltd.
BCR 701	Lake sediment	Sigma-Aldrich
ERM-CD281	Rye grass	Sigma-Aldrich
NCS DC73349	Bush, branches and leaves	LGC Standards

3.7.13 Low Molecular Weight Organic Acid Extraction

A low molecular weight organic acid extraction (LMWOA) was first suggested by Feng *et al.* (2005) where a 10mM total concentration of acetic, lactic, citric, malic and formic acids are combined at a ratio of 4:2:1:1:1 to represent the extraction potential of the rhizosphere environment via organic acids from soil organisms.

Extraction Procedure

Two grams of previously prepared sample (section 3.6.2) was accurately weighed into a 50 ml centrifuge tube (Falcon™, Sigma-Aldrich) with 20 ml LMWOA extractant. The sample was shaken for 16 hours on an end-over-end shaker at ~18°C before centrifugation at 3,000 x g⁻¹ for 10 minutes. The supernatant was decanted into a clean tube and analysed directly via ICP-OES (section 3.7.17) within three days.

3.7.14 Calcium Chloride Extraction

The use of a weak solution of CaCl₂ has been used extensively to provide an indication of plant-available elements in a soil environment, with several studies providing evidence of strong correlations of plant tissue concentrations and of a range of CaCl₂ – extractable PTE (Maiz *et al.* 2000; Meers, Samson, *et al.* 2007; van Gestel 2008).

Extraction Procedure

The extraction was completed based on Houba *et al.*, (2000) where one gram of previously prepared sample (section 3.6.2) was accurately weighed into a 50 ml centrifuge tube (Falcon™, Sigma-Aldrich) with 10 ml of 0.01 mol L⁻¹ CaCl₂

(≥99.9% trace metals basis, Sigma-Aldrich). The sample was shaken on an end-over-end shaker for 2 hours at 30 rpm and subsequently extracted by centrifugation for 8 minutes at 15,000 x g⁻¹. The supernatant was decanted into a clean tube, acidified with 100 µl of concentrated nitric acid (Aristar®, 69% w/v, VWR International Ltd) stored in the dark at 4°C before analysis using ICP-OES (section 3.7.17) within three days.

3.7.15 Partition Coefficients

Principal Concepts

Partition coefficients are based on the interchanges of analytes of interest from the solid to the aqueous phase, where water is in contact with the material in question. In terms of PTE mobility the concept is a simplified model where the concentration of an element of interest is measured in the solid phase and subsequently determined in a solution (usually water) after undergoing desorption processes. The model is detailed in (Equation 8) below. The principal aims to give a simplified ratio that allows a straightforward assessment of the retention capacity for soils contaminated with PTE.

$$K_d = \frac{\text{Total Metal Concentration (mg/kg)}}{\text{Dissolved Metal Concentration (mg/L)}}$$

Equation 8. Partition coefficient model.

Low ratios can be generally interpreted as demonstrating high desorption and therefore high potential mobility of an element in question in the environment. The K_d value will be constrained by the underlying environmental characteristics of the sample in question, with pH, organic matter and total elemental concentrations often demonstrated as the most significant controlling variables (Sauve *et al.* 2003).

Determination of K_d

Partition coefficients in this study were determined using solution concentrations of CaCl₂ extractions (section 3.7.14) of the 24 samples collected in section 3.2.4 in each respective tailing location. Calculated K_d coefficients were log transformed to allow comparison between sites.

3.7.16 Bureau of Community Reference Sequential Extraction

Sequential extraction of PTE is a useful tool for understanding the concentration of targeted elements in operationally defined soil fractions. The use of sequential extractions has allowed a greater understanding of the chemical behaviours of PTEs in the solid phase, their possible risks in the current environment and the ability to forecast potential changes of mobilisation and immobilisation in remediation efforts or changes to the soil environment. Over the past three decades several procedures have been developed e.g. (Tessier *et al.* 1979; Hall *et al.* 1996; Maiz *et al.* 1997), however several inconsistencies in the application of these methods promoted the development of a standardised extraction scheme by the EC Standards, Measurements and Testing Programme alongside a certified reference material, BCR-701® (Pueyo *et al.* 2001).

The basic principal of a sequential extraction is to subject a sample to increasingly aggressive extracting solutions and procedures and assess the concentration of target elements liberated from the solid phase into solution. For the BCR procedure, the three operationally defined fractions are as follows:

- i). Weakly absorbed, carbonate bound and acid soluble elements. This includes freely hydrated ions, those that are easily displaced from the solid phase and those that participate in reversible desorption/adsorption reactions immediately. This fraction is often of the most concern as it is associated with the greatest bioavailability and mobility of target PTE and is extracted using acetic acid.
- ii). Reducibly held and Fe/Mn bound elements. This fraction focuses on Fe/Mn held elements which are a significant pool of solid phase PTE due to the high affinity of Fe/Mn (oxy)-hydroxides for divalent cations. The elements contained in this fraction are considered less mobile than fraction (i), however large releases of PTE may occur as a result of change in redox conditions (e.g. severe soil flooding). For this fraction hydroxylamine hydrochloride is used as an extractant.

- iii). Organically and sulphite bound elements. This fraction is considered to be mostly unavailable for biotic interaction and mobility in the soil environment due to the chemically stable nature of sulphites and strongly bound organo-metallic compounds, with minimal environmental release from these compartments occurring over a period of decades. This fraction is liberated using hydrogen peroxide to promote the dissolution of organic and sulphite bound structures and ammonium acetate to dissolve the released elements into the analytical solution.

For completeness, a final stage to the BCR procedure is often added to determine the proportion of PTE that are held in chemically resistant, immobile phases. This fraction is termed the residual phase and chemical release from this compartment is highly unlikely under normal environmental conditions, with substantial weathering of the mineral structure required over a long period of time necessary to remove the elements bound in this pool. For this fraction, strong acidic approaches such as *aqua-regia* and nitric-hydrofluoric acid combinations are necessary to dissolve the held PTE.

Despite the clear advantages in applying a sequential extraction and the depth of detail that can be recovered the procedures have been subject to several caveats, such as the inherent errors when calculating repeat extractions of a single sample, the dissolution of non-target phases, reabsorption and redistribution of liberated elements and the loss of sample due to operator error (Bacon and Davidson 2008). Therefore, caution should be exercised when undertaking sequential extraction procedures and it is essential that quality control measures are integrated into throughout the procedure.

Sequential Procedure

The extraction was conducted strictly adhering to (Pueyo *et al.* 2001) in exception to the volume of centrifuge tubes, where 50 ml tubes were substituted for the stated 100 ml. The procedure was completed over three consecutive days to reduce issues relating to sample residues changing after exposure to the previous extractant.

Fraction i)

One gram of previously prepared sample (section 3.6.2) was accurately weighed into a 50 ml centrifuge tube (Falcon™, Sigma-Aldrich). To this, 40 ml of 0.11 mol L⁻¹ acetic acid (99.7% Fisher) was added and the sample immediately placed onto an end-over-end shaker at 30 rpm for 16 hours at 20°C. After the shaking period the sample was immediately centrifuged at 3,500 x g⁻¹ for 20 minutes and the supernatant decanted into a clean 50 ml centrifuge tube, taking care to retain the sample residue. The supernatant was stored at 4°C in the dark.

The remaining residue was washed by introducing 20 ml of de-ionised water to the centrifuge tube, re-suspending the sample and placing on an end-over-end shaker at 30 rpm for 15 minutes. The sample was again centrifuged for 20 minutes at 3,500 x g⁻¹ and the washings discarded, again taking care to retain the solid phase.

Fraction ii)

The residue from fraction (i) was exposed to 40 ml of 0.5 mol L⁻¹ hydroxylamine hydrochloride (≥96 %, Sigma-Aldrich) (pH adjusted with 25 ml 2 mol L⁻¹ nitric acid (Aristar®, VWR) and processed following the same procedure as fraction i).

Fraction iii)

To the residue from fraction (ii) 10 ml of 30% w/v hydrogen peroxide (Fisher) was added and the sample manually shaken to ensure thorough mixing. The samples were digested for one hour at room temperature (20-23°C) with occasional manual shaking, releasing pressure if required from the tubes. After ambient digestion the samples were transferred to a water bath (Fisher, Model 202) and digested at 85°C for one hour with occasional shaking for the first 30 minutes, leaving the centrifuge tubes slightly uncapped to release excess pressure. After this stage the samples were uncapped and the digestion solution evaporated until approximately 3 ml remained, with a further 10 ml of 30% w/v hydrogen added to the sample and digested at 85°C following the previous procedure. The samples were evaporated for a second time until approximately 1 ml of solution remained.

Once cooled, 50 ml of 1 mol L⁻¹ ammonium acetate ($\geq 98\%$, Sigma-Aldrich) buffered to pH 2 using conc. nitric acid (68%, Aristar[®], VWR) was added to the residue and extracted following the same methodology as fraction (i) and (ii).

Extraction of the Residual Phase

The residue from the sequential extraction was transferred quantitatively to a clean beaker using deionised water to wash the centrifuge tube. The slurry was gently evaporated at 40°C until dryness was achieved. From this an *aqua-regia* extraction was performed.

To the dried residue 1 – 2 ml of water was added to form a slurry and subsequently 30 ml of *aqua-regia* (3:1 v/v conc. hydrochloric (37%, Aristar[®], VWR) and conc. nitric acid (68%, Aristar[®], VWR) was added to the sample. The sample was refluxed on a hotplate (Stuart, CB-500) for two hours, allowed to cool and filtered through a Whatmann 540 hardened, ashless filter paper (SigmaAldrich) into a clean beaker. The sample was placed back on the hotplate and concentrated via evaporation until approximately 1-2 ml remained. The sample was then quantitatively transferred to a 25 ml volumetric flask, washing the beaker with 2% v/v nitric acid and made up to the mark accurately with 2% v/v nitric acid before decanting into a 30 ml polyethylene steralin tube (Thermo Scientific) in the dark at 4°C until analysis.

Quality Control

In each batch run two one-gram replicates of BCR-701[®] was included plus two blanks of each extractant solution. Additionally, the final stage involving extraction via *aqua-regia* also included two replicate samples of 1 g, CRM 7002. The vessels containing the blanks were retained throughout the extraction procedure to identify if extraction solutions released contaminants from the centrifuge tubes. Separate blanks from the extractant solution were assessed alongside the process blanks to identify if extraction solutions were a source of elemental contamination.

In addition, a separate, ashed (450°C, 4 hours) 0.25 g replicate sample was extracted directly with 30 ml *aqua-regia* to verify the sequential extraction recoveries.

Matrix matched calibration standards were created using reagents of a higher quality than the extractant solutions (ammonium acetate extra pure, (EMD Millipore), hydroxylamine hydrochloride (99.9995, trace metals basis, Sigma-Aldrich) acetic acid and hydrogen peroxide (Aristar®, VWR) to reduce issues with suppressing elemental contamination identification in the analysed samples.

3.7.17 Elemental Analysis via Inductively Coupled Plasma Optical Emission Spectroscopy.

Method Principal

Prior to the advancement of atomic spectroscopy techniques elemental analysis was mainly conducted through complexation reaction and the measurement of coloured solutions by UV-spectroscopy. These methods, whilst cheap, were often time consuming, constrained in scope by high limits of detection and could only analyse a single element per method. With advances in spectroscopic techniques several technologies were developed that enabled greater resolution of analysis and the ability for simultaneous, multi-elemental quantification.

Inductively coupled plasma optical emission spectroscopy (ICP-OES) uses a spark from a Tesla coil to produce an electrically conductive, gaseous flow of electrons and cations that can reach temperatures of up to 10,000 K, with the concentration of cations and electrons in the plasma producing a net overall charge to the matter. In most systems, argon is used as a source of cations and electrons due to the relatively low cost, low ionisation energy and low potential for forming oxide species with other ions.

Samples are most commonly introduced in an aqueous form, with target elements either dissolved or suspended in solution. The sample is conveyed to the plasma torch via a nebuliser which promotes the transformation of the solution to an aerosol, which is subsequently swept into the plasma source.

When a sample is introduced to the plasma the elements in the solution undergo atomisation and then ionisation when passing through the energy source. In this process, an electron in the atom is promoted from its ground state into a higher

orbital via excitation and upon exiting the high energy environment, the electron relaxes to its original orbital and releases the absorbed energy as a photon. Each element emits photons at several characteristic wavelengths which can then be divided via a polychromatic separator and analysed using photo-responsive detectors such as a photo-multiplier tubes, charged injection devices or charged coupled devices. From this the concentration of an element can be analysed, as the intensity of light at a given wavelength is proportional to the concentration of the element of interest. This quantification is based on previous calibrations of known concentrations of the target element and normally, the use of linear regression to determine unknown sample concentrations (Skoog *et al.* 2014).

The overall process of ICP-OES is presented diagrammatically in Figure 3-12.

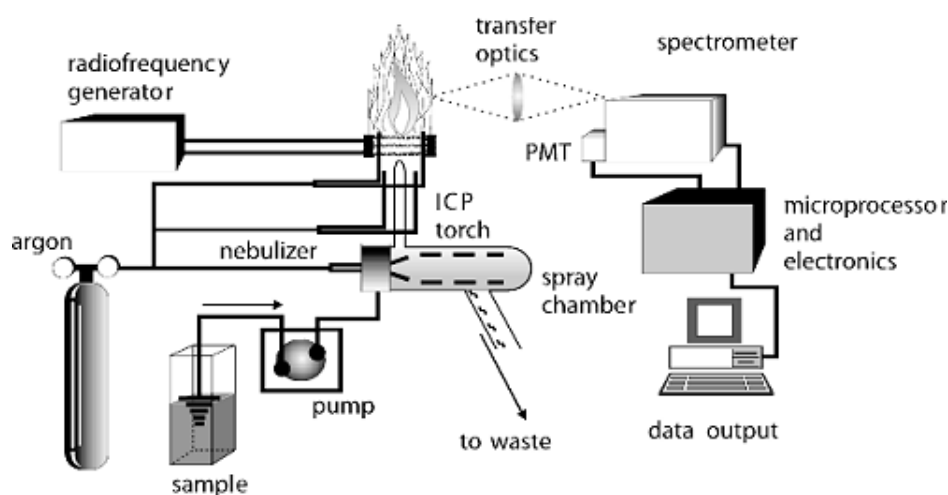


Figure 3-12. Schematic of ICP-OES principle.

ICP-OES Analysis Procedure

Samples were processed on a Perkin-Elmer Optima 8300DV ICP-OES instrument, using a Scott-type nebuliser with gem-tips and AS 90 plus auto-sampling system. The system was operated in axial mode on all investigations with plasma, auxiliary and nebuliser argon flow rates set at 10, 0.2 and 0.8 L min⁻¹ respectively. Operating conditions are detailed in Table 3-10.

Table 3-10. Operating parameters of the Perkin Elmer Optima 8300 DV ICP-OES.

Parameter	Value
Radio Frequency Power	1500 W
Nebuliser and Spray Chamber	Scott Type, gem tip cross-flow
Argon Flow Rates	Plasma – 8, Auxiliary - 0.2, Nebuliser – 0.6 L min ⁻¹
Peristaltic Pump Flow	1.0 ml min ⁻¹
Viewing mode	Axial

Preparation of ICP-OES Calibration Standards

Calibration standards were created using either single 1000 mg L⁻¹ elemental standards (SCP Science) or ICP multi-element standard solution IV (Merck) depending on the range and choice of elements for each analytical run. A separate set of standards were used for elements where issues with precipitation of Ag in the multi-elemental standard were encountered (e.g. As and P standards were in a HCl matrix). Calibration ranges differed depending on the analytical procedure but contained at least seven calibration points in each run, with standards freshly made immediately before ICP-OES operation.

In each analytical case, standards were matrix matched with the extractant solution to minimise analytical issues and matrix induced interferences. The wavelengths used to measure elemental concentrations are detailed in Table 3-11.

Table 3-11. Wavelengths monitored during ICP-OES analysis with manufacturer's and user limits of detection.

Element	Wavelengths Used	Manufacture's Detection Limits ($\mu\text{g/L}^{-1}$)	User's Detection Limits ($\mu\text{g/L}^{-1}$) *
Ag	328.068, 338.289, 243.778	0.6	N/A
Al	394.401, 308.215, 396.153	1	211
As	188.979, 193.396, 197.197	1	2.7
B	249.677, 248.772, 208.889	1	662
Ba	233.527, 455.403, 493.408	0.03	5.59
Bi	223.061, 190.171, 306.766	1	7.41
Ca	317.933, 315.887, 393.366	0.05	57
Cd	228.802, 214.440, 226.502	0.1	7
Co	228.616, 238892, 230.786	0.2	0.52
Cr	267.716, 205.560, 283.563	0.2	4.07
Cu	327.393, 324.752, 224.700	0.4	0.95
Fe	238.204, 239.562, 259.939	0.1	97
Ga	417.206, 294.364, 209.134	1.5	1.7
In	230.606, 325.609, 303.936	1	0.055
K	766.490, 404.721	1	58
Li	670.784, 610.362, 460.286	0.3	0.13
Mg	285.213, 279.077, 279.533	0.004	20
Mn	257.610, 259.372, 260.568	0.005	0.15
Na	589.582, 330.237, 588.995	0.5	472
Ni	231.604, 221.648, 232.003	0.5	221
P	213.617, 214.914, 178.221	4	7.8
Pb	220.353, 217.000, 261.418	1	6.42
S	181.975, 180.669, 182.563	10	5.69
Sb	206.836, 217.582, 231.146	2	2.24
Sr	407.771, 421.552, 460.733	0.05	0.051
Ti	334.940, 336.121, 334.903	0.4	4.29
Tl	190.801, 276.787, 351.924	2	11.90
Zn	206.200, 213.857, 202.548	0.2	0.031

* User detection limited are based on the highest overall detection limit established on the instrument across all matrices analysed during the investigation. Where lower concentrations are stated in the results the specific LOD will also be provided.

Quality Control

In each analytical run calibration drift standards and blanks were included every 10 samples and at the end of each analysis. In addition, an initial check using an independent, matrix matched ICP multi-element standard solution VI (Merck) diluted in a 1:10 ratio was incorporated at the beginning and the end of the analytical run to assess accuracy and drift of the analytical process. In the event of instrument drift or unacceptable measurement error the instrument was corrected and the samples re-run.

3.7.18 Inductively Coupled Plasma Mass Spectroscopy

Method Principal – Quadrupole System

Inductively coupled plasma mass spectroscopy (ICP-MS) uses the same ionisation approach as the ICP-OES (section 3.7.17) to convert elements that are suspended or dissolved in solution into ions that can be determined by the instrument. The ICP-MS differs in the detection method from the ICP-OES where elements are separated by their mass-to-charge ratio (m/z), rather than the emission of photons.

In the ICP-MS, ionised atoms are then passed through an interface consisting of two cones with a small entrance hole in order to sample the centre of the ion beam and transfer the ions from an atmospheric pressure to the low-pressure region of the mass spectrometer. The ions are then focused by lens discrimination, where the ion beam is collimated (narrowed) and separated from unwanted neutral species and photons by electrostatic lenses. The focused ions are then introduced to the quadrupole system, in which four rods of two pairs aligned oppositely to each other utilise rapidly changing radio frequency (RF) and direct current (DC) potentials to create an electrostatic filter, allowing only ions of a specific m/z to pass into the detection system at a given time. Changes in the RF/DC parameters allow either continuous scanning across the atomic mass unit or rapid switching between target m/z . Finally, the positively charged ions collide with a detection system consisting of a highly charged negative surface, releasing the electrons which amplifies the signal upon contact with the next detection layer. This electrical signal is then translated into elemental concentrations when compared to signals from a previously introduced reference standard.

The overall principal of ICP-MS is graphically demonstrated in Figure 3-13.

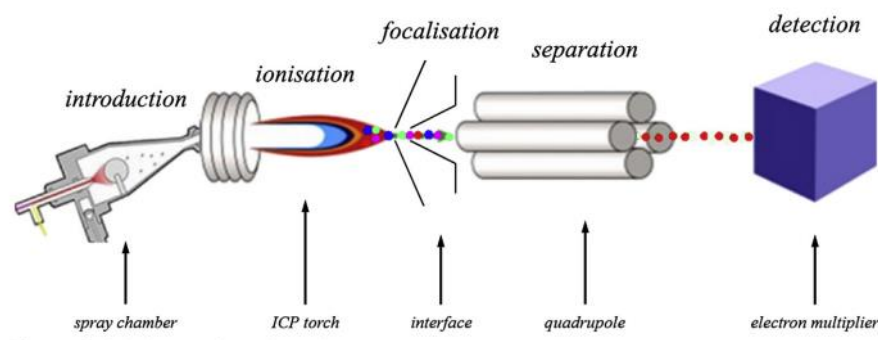


Figure 3-13. Principal of ICP-MS analysis.

ICP-MS Analysis Procedure

In this investigation an Agilent 7,500ce quadrupole mass analyser was used in conjunction with a conventional Agilent octapole detection system, integrated autosampler (I-AS), Ni cones and a Scott-style spray chamber micro mist nebuliser system. Operational parameters are detailed in Table 3-12.

Table 3-12. Operational parameters of ICP-MS analysis of aqueous samples.

Parameter	Value
Radio Frequency Power	1540 W
Carrier Gas Flow (Argon)	0.81 L/min ⁻¹
Make-up Gas Flow (Argon)	0.22 L/min ⁻¹
Nebuliser uptake rate	L/min ⁻¹
Acquisition Mode	Spectrum
Analyser Pressure	3.4 x 10 ⁻⁶ Pa
Rinse speed, uptake time	0.3 rps, 30 seconds
Peak Pattern	3 – Fully Quantitative
Rinse time (2% HNO ₃)	10 seconds

Calibration and Quality Control

For the analysis of river samples (section 3.6.5) the following isotopes and LOD were used for the determination of Cd, Pb and Zn (Table 3-13). Calibration standards consisting of 0, 0.1, 0.5, 1, 5, 10, 50, 100 and 250 µg L⁻¹ were produced by serial dilution of a freshly made 1,000 µg L⁻¹ stock solution derived from single 1000 mg L⁻¹ elemental standards (SCP Science). In addition, SRM 1640a (NIST) was used as an independent verification material of instrument accuracy at the beginning and end of each run. Each analytical run also included a calibration drift and blank check at least every 5 samples.

Table 3-13. Elements and atomic masses of analytes determined using ICP-MS.

Element	Atomic Mass Unit(s) Examined	Limit of Detection ($\mu\text{g/L}^{-1}$)
Cd	111	0.06
Pb	64, 66, 67, 68	0.1
Zn	208	1.1

3.7.19 X-Ray Diffraction

Method Principal

X-ray diffraction (XRD) uses electromagnetic radiation between 10 and 0.01 nm to determine the crystalline structure of minerals in a solid matrix. Generated x-rays directed towards a sample interact with the matrix, producing interferences that can be interpreted using Bragg's Law. The diffracted radiation can be detected over a range of angles, allowing for lattice spacing, direction of diffraction and wavelengths to be assessed by the instrument. These diffraction qualities can then be related to specific minerals as each mineral component in a sample exhibits a unique spacing of atoms, and thus will produce different diffraction patterns.

A powdered sample is essential for XRD processing as the sample must represent all the individual orientations of each crystal present to allow for accurate detection as well as producing a random distribution of the crystals in the surface.

XRD Procedure

XRD analysis was completed on a Bruker D8 Advance with Sol-X Energy Dispersive detector after samples were prepared following section 3.6.2. Crystal identification was achieved using Bruker Diffrac.EVA software and the current International Centre for Diffraction Data (ICDD) database.

3.8 Additional Calculations

3.8.1 Limit of Detection and Limit of Quantitation

The limits of detection (LOD) and the limits of quantitation (LOQ) for all analytical procedures were calculated based on (IUPAC 1978; MacDougall *et al.* 1980), The limit of detection is defined as the smallest concentration that can

be reliably detected with a reasonable degree of certainty, whereas the LOQ is termed as the concentration of which can be assessed with a high degree of confidence by the procedure in question. The LOD and LOQ are determined following Equation 9 and Equation 10 respectively

$$LOD_{(concentration)} = \frac{3 \times Std\ Dev.\ Calibration\ Blank}{Calibration\ correlation\ coefficient}$$

Equation 9. Calculation used to quantify the limit of detection.

$$LOQ_{(concentration)} = \frac{10 \times Std\ Dev.\ Calibration\ Blank}{Calibration\ correlation\ coefficient}$$

Equation 10. Calculations used to quantify the limit of detection and limit of quantitation.

3.8.2 Elemental Concentrations of Acid Digested and Solution Extracted Samples

Elemental concentrations of environmental samples digested following section 3.7.12 and extractions following sections 3.7.13 and 3.7.14 were analysed via ICP-OES (section 3.7.17) were determined following Equation 11.

$$Concentration\ (mg/kg) = \frac{ICP - OES\ concentration\ (mg/L) \times Extractant\ volume\ (L)}{Mass\ of\ sample\ (kg)}$$

Equation 11. Calculation for the determination of elemental concentrations in solid environmental samples

3.8.3 Bioaccumulation Coefficient

This calculation considers the metal concentration in plant tissues and PTE concentrations in the tailing material (Equation 12). This determination allows inferences for the ability of plants to absorb PTE from the solid phase.

$$Bioaccumulation\ coefficient = \frac{Plant\ PTE\ concentration\ (mg/kg)}{Solid\ phase\ PTE\ concentration\ (mg/kg)}$$

Equation 12. Bioaccumulation coefficient calculation.

3.8.4 Translocation Factor

The capacity of vegetation to translocated absorbed PTE from the root to the shoot is described in Equation 13.

$$\text{Translocation factor} = \frac{\text{Concentration in shoot (mg/kg)}}{\text{Concentration in root (mg/kg)}}$$

Equation 13. Calculation of plant translocation capacity.

3.8.5 PTE Extraction Efficiency

The efficiency of a plant to remove PTE from soil is given in Equation 14.

$$E = \left[\frac{(\text{Contaminated soil} - \text{Contaminated soil under phytoremediation})}{\text{Contaminated soil}} \right] \times 100$$

Equation 14. Calculation of plant PTE extraction efficiency.

Chapter 4. Site Characterisation and Kriging

4.1 Introduction

This chapter gives an overview of the elemental concentrations and distributions at the Tyndrum (W.Scotland) and Le Bleymard (S.France) Pb/Zn mines, with specific focus on Cd, Pb and Zn. To support the characterisation of the site plant species, tailing characteristics and surface water concentrations of these elements were assessed to give a comprehensive understanding of the sites. A range of field and laboratory results were then used to measure the baseline characteristics of the mine tailings, and results were then presented using geo-statistical techniques. This allowed for spatial inferences to be made together with identification of areas that may present point source pollution and barriers to remedial approaches. These assessments provide better understanding of the processes that may be driving elemental distribution and vegetation growth on the sites.

4.2 Rationale and Overview of Chapter

Understanding the distribution and concentrations of PTEs and tailing characteristics is an important prerequisite to understanding the mechanisms of possible pollution pathways, potential risks to receptors and assessing the barriers or challenges to potential remediation strategies. The initial site survey of both the Tyndrum and Le Bleymard tailings was used to inform chapters 5, 6 and 7 in this thesis, based on the chemical, biological and physical findings in this section, as well as contributing to the existing literature on these mine tailings. In particular, this is the first time either mine tailings have undergone a detailed spatial survey of the surface layer (0-20 cm), highlighting the complexity of the tailing areas and identifying distributions of PTEs and other tailing characteristics.

4.3 Brief Introduction to Tyndrum and Le Bleymard

The historical background of both mines has been explored in detail in Chapter 2. The mine tailings at Tyndrum comprise of a 1.1 ha area, located in the west coast of Scotland in the Loch Lomond and Trossachs National Park (NN 32174 30695). The mine has undergone sporadic and uncontrolled mining for over 200

years until 1928 (Mills *et al.* 2014), resulting in visible scarring to the landscape and the enrichment of sediments in Loch Tay with Pb, approximately 25 km downstream (Farmer *et al.* 1997).

The mine tailings at Le Bleymard are located in The Cévenne National Park in France (44°28'23.0"N, 3°43'42.5"E), with pollution events linked to the airborne dispersion of the tailing material (Ploquin 2010) and elevated concentrations of Cd in wild brown trout within the catchment area (Monna *et al.* 2011). The mine has undergone commercial mineral extraction since 1903, with abandonment in 1953 (Ploquin 2010).

4.3.1 Methods and Data Analysis

Tailing Sampling

Twenty-four tailing samples were obtained using a stratified random strategy (Chapter 3, section 3.2.4). A composite tailing sample was recovered using an auger and placed into a labelled polyethylene bag. Composite tailing samples were created by taking three individual tailing samples in a triangular formation approximately 10 cm distance apart and combining the sample in the same bag to produce a representation of approximately 200 g of the tailing material.

Tailing samples were assessed for total and CaCl₂ extractable PTE concentrations, pH, TN/TC, particle size and OM content (Chapter 3. Sections 3.6 and 3.7). Empirical Bayesian Kriging (EBK) and Inverse Distance Weighting (IDW) were applied to interpret the geo-spatial distribution of the measured variables in Le Bleymard and Tyndrum respectively (Chapter 3. Section 3.2.3) in ArcGIS 10.1. Other graphical and statistical outputs were completed using Origin® 2016.

Surface Water Samples

Surface water samples were taken periodically throughout the investigation to attempt to account for variations over time and possibly seasonality (Chapter 3, sections 3.2.5 - 6). At each sampling period duplicate samples were taken at each pre-determined point, acidified, filtered and analysed following Chapter 3, section 3.7.18. An un-acidified aquilot was used for the determination of pH

following section 3.7.2. Graphical and statistical outputs were completed using Origin® 2016.

Vegetation

Vegetation was surveyed via three random quadrats at each tailing sampling point following NVC protocol (Rodwell 2006) (Chapter 3, section 3.2.2). No vegetation was removed from the site. Areas of vegetation were digitised using ArcGIS 10.1 based on recent satellite imagery (Google Earth 2016) for Le Bleymard and aerial imagery captured using an unmanned aerial vehicle at Tyndrum, courtesy of Simon Gibson-Poole, SRUC. Vegetation was surveyed in May 2015 and 2016 for the Tyndrum site and in April 2015 and August 2016 at the Le Bleymard mine (see Table 4-1) to account for seasonality due to the montane climate and late emergence of vegetation.

Overall Sampling Regime

Table 4-1. Overall sampling regime for surface waters, tailings and vegetation.

Parameter	Location	Sampling Period
Tailings	Tyndrum	May 2015
	Le Bleymard	April 2015
Surface Water	Tyndrum	May 2015
	Le Bleymard	April 2016, April 2017, October 2017
Vegetation	Tyndrum	May 2015 May 2016
	Le Bleymard	April 2015 August 2016

4.4 Results – Tyndrum

4.4.1 Physical Characterisation of the Tailings.

The physical description of a single profile pit, located in the centre of the tailings, is detailed in Table 4-2. The tailings comprise of a layer of fine sandy material of variable depth across the site that has the physical characteristics of a sandy soil. Several layers exhibited grey and orange mottling, suggesting periodic anaerobic conditions and gleying processes. No macro invertebrates or root systems were noted throughout the profile.

The overall observations suggest freely draining conditions to a depth of around 60 cm, thereafter visible evidence of anaerobic conditions was observed

Table 4-2. Physical description of a single pit at the Tyndrum Pb/Zn mine.

Horizon	Depth (cm)	Structure	Texture
1	0-50	Single grain, very fine, loose	Sand
2	50-65	Friable	Sand
3	65-75	Coarse, sub angular blocks	Sandy clay (visible mottling and gleying)
4	75-90	Coarse, angular, blocky, friable	Sandy clay
5	90-104	Medium, angular blocky, friable	Silt

4.4.2 Concentrations and Distribution of PTEs in the Mine Tailings

The concentrations of total PTE (s.d) are detailed in (Table 4-3) Concentrations of PTE are highly variable across the tailing area, with Pb and Zn notably elevated in the tailing material in comparison to environmental quality standards (EQS) and other PTEs such as Cu, Cd and As (Table 4-3). Calcium chloride extractable concentrations of Pb, Cd and Zn were approximately 4.3, 13.6 and 6.6 % of the mean total concentration respectively (Table 4-3)

Table 4-3. Mean total and CaCl₂ extractable concentrations of selected PTE at the Tyndrum Pb/Zn mine tailings. n = 24. LOD = limit of detection. Elevated concentrations of PTE highlighted in bold. *Environmental Quality Standards are based on soil concentrations for land deemed for industrial use (CCME 2018).

Element	Total Concentration (mg kg ⁻¹) (± std. dev)	Environmental Quality Standard* (mg kg ⁻¹)	CaCl ₂ Concentration (mg kg ⁻¹) (± std. dev)
As	18.49 (± 16.8)	12	< LOD
Cd	26.28 (± 19.1)	22	3.6 (± 4.5)
Co	9.663 (± 5.9)	300	0.36 (0.34)
Cr	19.22 (± 86.6)	87	3.1 (± 2.2)
Cu	534.3 (± 478)	91	2.1 (± 1.7)
Ni	36.05 (± 32.8)	89	0.6 (± 0.2)
Pb	12,261 (± 10,182)	600	527 (± 492)
Sb	5.08 (± 2.9)	40	<LOD
Zn	4,048 (± 3,531)	410	266 (± 198)

The interpolated spatial distribution of Pb, Zn and Cd were (Figure 4-1, 2 and 3 respectively) was found to vary significantly over small interpolated distances (< 5 m) and did not appear to show recognisable trends in terms of dispersion throughout the site. Total Pb was not correlated to other measured characteristics in the tailing material, however there was evidence of a higher concentration of Zn in the centre of the tailings (Figure 4-2). Zinc was correlated with total S ($R^2 = 0.72$) in the tailings, as was Cd.



Figure 4-1. Inverse distance weighting interpolation of total Pb concentrations at the Tyndrum Pb/Zn mine.

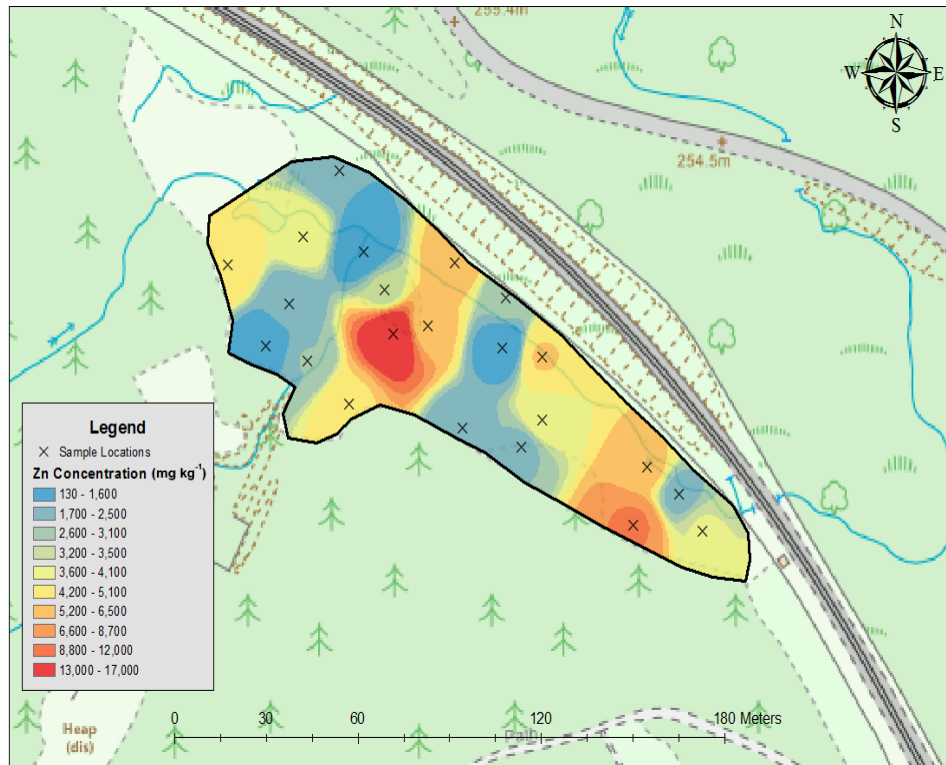


Figure 4-2. Inverse distance weighting interpolation of total Zn concentrations at the Tyndrum Pb/Zn mine.



Figure 4-3. Inverse distance weighting interpolation of total Cd concentrations at the Tyndrum Pb/Zn mine.

4.4.3 Partition Coefficients for PTE in the Tyndrum Mine Tailings.

Partition coefficients for the Tyndrum tailing material for Pb, Zn and Cd were highly variable (Table 4-4), with evidence to support both high and low mobility in the tailing material. Partition coefficients for Pb were strongly correlated with pH (Figure 4-4), indicating localised pH in the tailing to be a strong driver of desorption and potential mobility of Pb. In contrast, Cd and Zn K_d coefficients were not correlated to pH, particle size, total concentration or other measured variables, suggesting influence of other drivers, possibly due to implications with ‘aging’ associated with Zn and complexation of Cd onto spoil surfaces.

Table 4-4. Partition Coefficients for Pb, Zn and Cd at the Tyndrum Pb/Zn tailings.

Element	Log K_d Coefficient	Min	Max
Pb	2.9	1.9	3.9
Zn	2.4	1.7	2.9
Cd	2.0	1.4	2.9

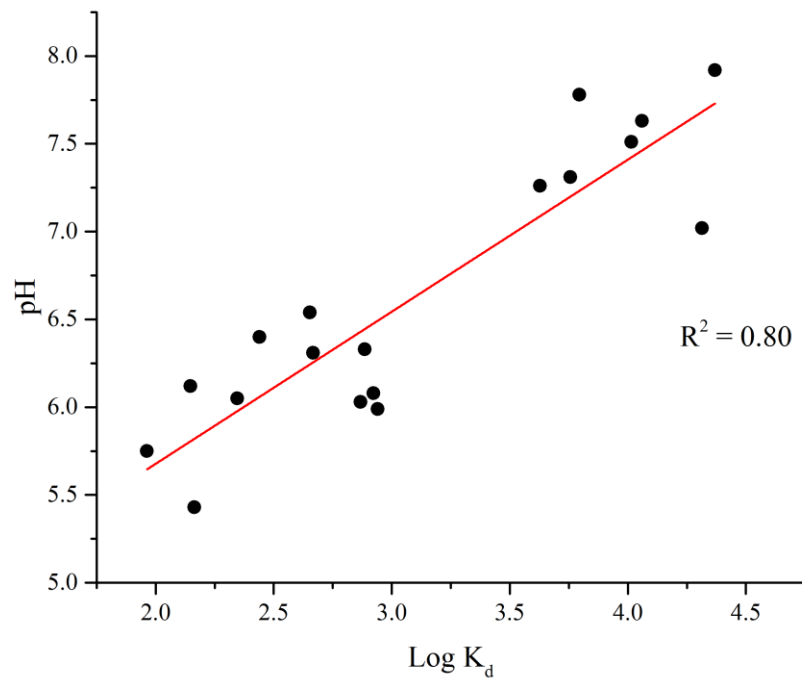


Figure 4-4. Correlation between log-transformed Pb K_d values and tailing pH at the Tyndrum Pb/Zn mine.

4.4.4 Tailing Characteristics in the Mine Tailing Site

Measured tailing characteristic parameters are found in Table 4-5. In general, the tailings exhibit a weakly acidic and skeletal system, with low concentrations of TN, TC and OM. Similarly to PTE concentrations, the site is highly heterogeneous when considering the distribution of measured variables on site. Concentrations of Mn, Ca, Fe and S are not found to be particularly different from expected global values (Hooda 2010), suggesting that plant deficiencies of these elements would not be expected to be significant.

Table 4-5. Values of selected tailing parameters at the Tyndrum lead mine tailing. n = 24

Parameter	Value (\pm std. dev)
pH	5.5 (\pm 0.8)
Total N (%)	0.18 (\pm 0.3)
Total C (%)	5.2 (\pm 7.5)
Loss on ignition (%)	11.05 (\pm 18.9)
Total Ca (mg kg ⁻¹)	1,255 (\pm 1,588)
Total P (mg kg ⁻¹)	688 (\pm 657)
Total Fe (mg kg ⁻¹)	23,306 (\pm 10,063)
Total Mn (mg kg ⁻¹)	502 (\pm 569)
Total Mg (mg kg ⁻¹)	3,028 (\pm 3,668)
Total S (mg kg ⁻¹)	4,035 (\pm 2,296)
Median Particle size range (μ m)	37 - 630

Total N was variable across the mine tailings, with elevated presence spatially linked to the presence of vegetation (Figure 4-5). Higher concentrations of TN were generally found on the boundaries of the tailings and lower presence was generally observed in the centre. Similarly, higher TC concentrations were linked with vegetation presence (Figure 4-6) with a deficit of TC generally found in the south eastern section of the tailings.

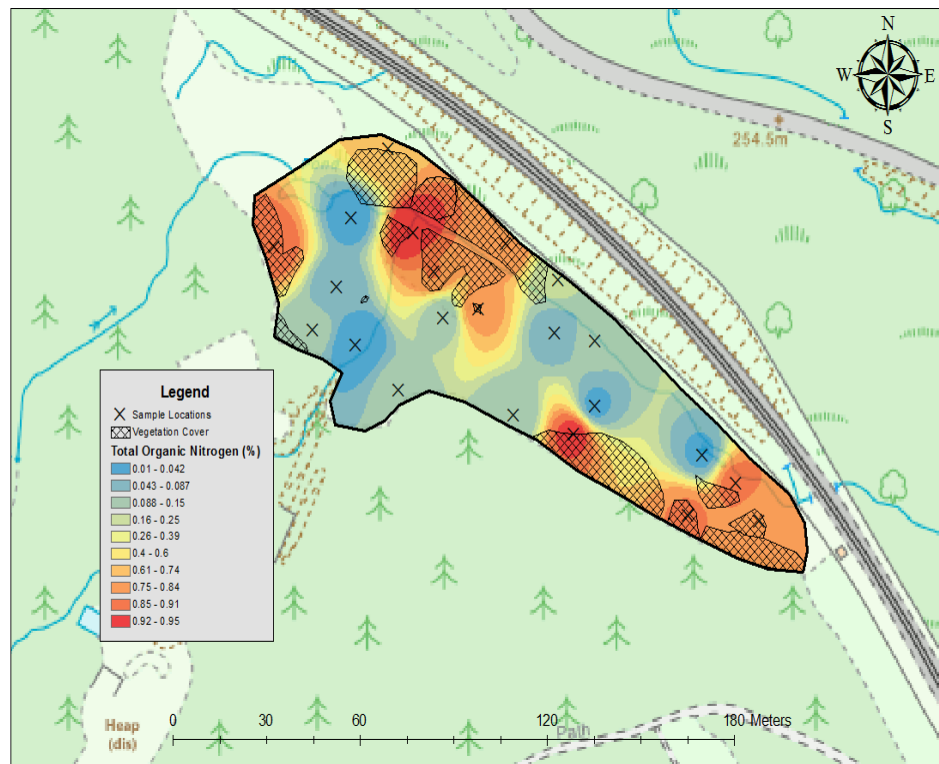


Figure 4-5. Inverse distance weighting interpolation of total nitrogen at the Tyndrum Pb/Zn mine.

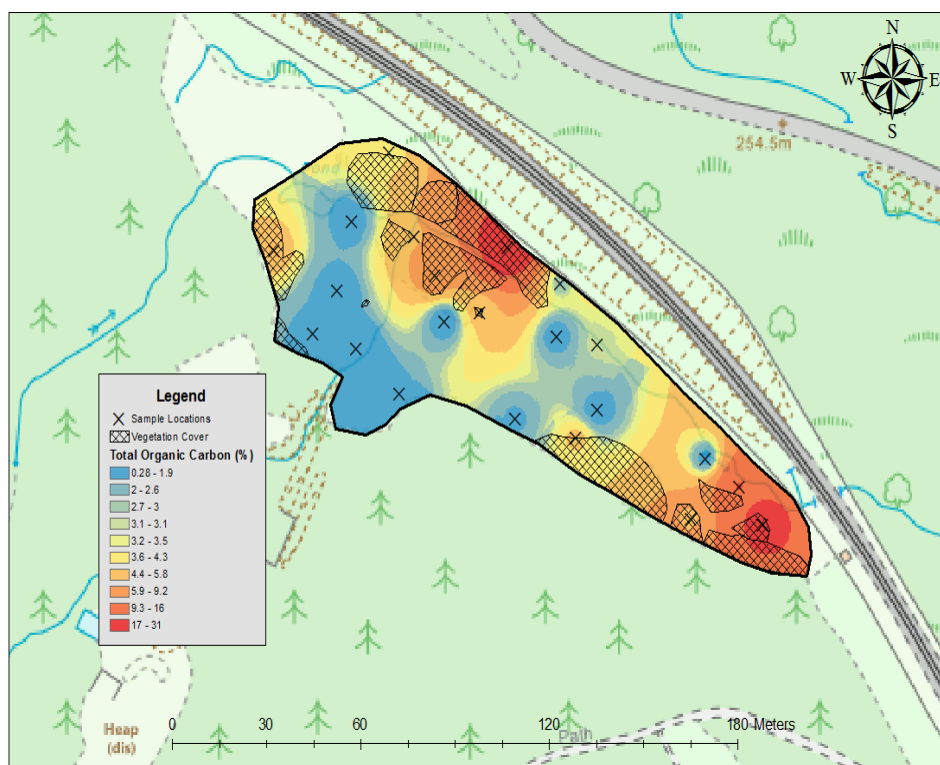


Figure 4-6. Inverse distance weighting interpolation of total carbon at the Tyndrum Pb/Zn mine.

The pH of the tailing material was observed to be highly heterogeneous across the site (Figure 4-7), with no correlations to measured site characteristics (in the exception of Pb - K_d), a weak spatial correlation to vegetation cover, however may exist.

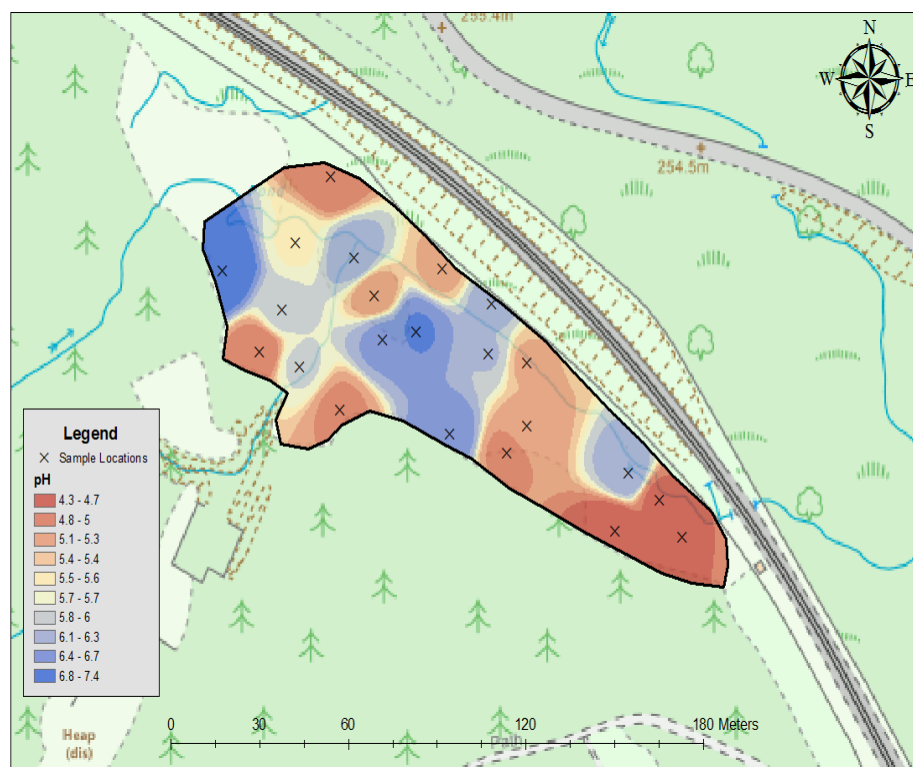


Figure 4-7. Inverse distance weighting of tailing pH of the Tyndrum Pb/Zn tailings.

4.4.5 X-ray Diffraction of Tyndrum Tailing Material

X-ray diffraction (XRD) (Method section 3.7.19) revealed a range of Pb and Zn containing minerals at the Tyndrum tailings, mainly anglesite (PbSO_4), galena (PbS), lead oxide carbonates ($\text{PbCO}_3\text{-PbO}$ and $\text{PbCO}_3\text{-2PbO}$) sphalerite (ZnFeS) and smithsonite (ZnCO_3) (Figure 4-8). The tailings exhibit several secondary, weathered mineral phases in the form of carbonates. The presence of chalcopyrite (CuFeS_2) was also found upon XRD analysis.

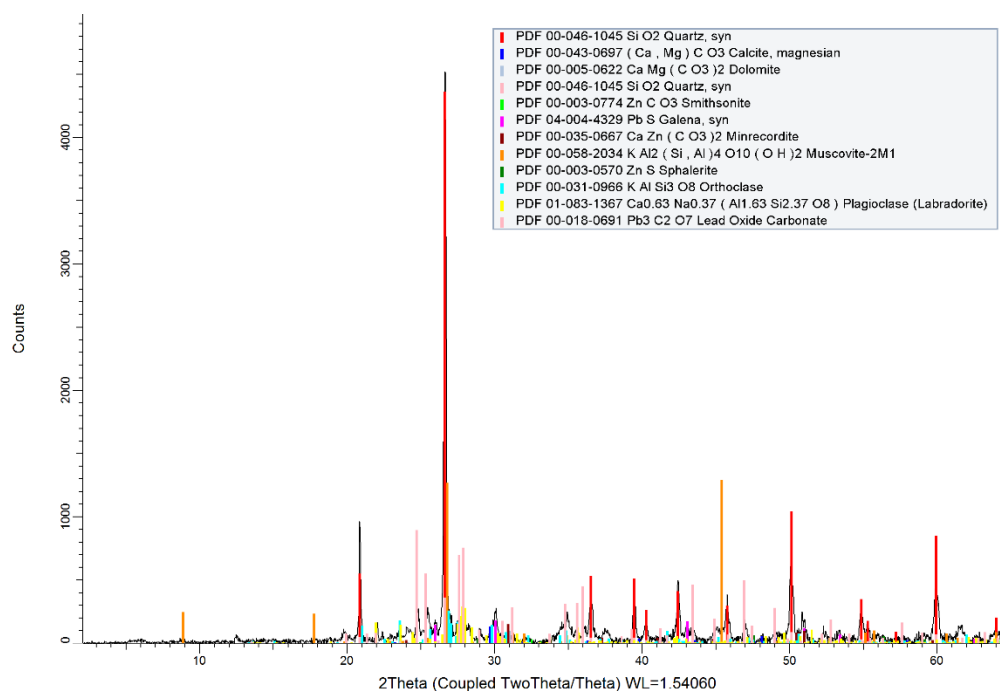


Figure 4-8. X-ray diffraction of a typical sample sourced from the Tyndrum Pb/Zn mine tailings.

4.4.6 Plant Species on the Tyndrum Mine

Plant species found on the mine were predominantly from the grass and heather families, namely *C.vulgaris*, *A.capillaris*, *A.vinealis* and *F.ovina*. Vegetation coverage was highly variable with ranges between 0-90% depending on the species and location of sample. As previously noted, vegetation cover was spatially linked to higher TN and TC concentrations.

4.4.7 Waterbody Concentrations of Cd, Pb and Zn

Concentrations of dissolved Pb and Zn collected from the fresh water stream were between 1,800 – 3,000 and 1,800 – 1,900 $\mu\text{g L}^{-1}$ respectively, within the immediate tailing area (Figure 4-9). Concentrations of Pb and Zn declined with distance, with a significant difference found after 300 m ($p = < 0.001$) at a sampling point immediately below a small tailing dam. After approximately 300 m, Pb concentrations were significantly lower than those of Zn ($p = < 0.0001$), with both elements demonstrating stable concentrations in the water column. The pH of the water column increased with distance from the tailings, with a notable increase at approximately 300 m (Figure 4-10).

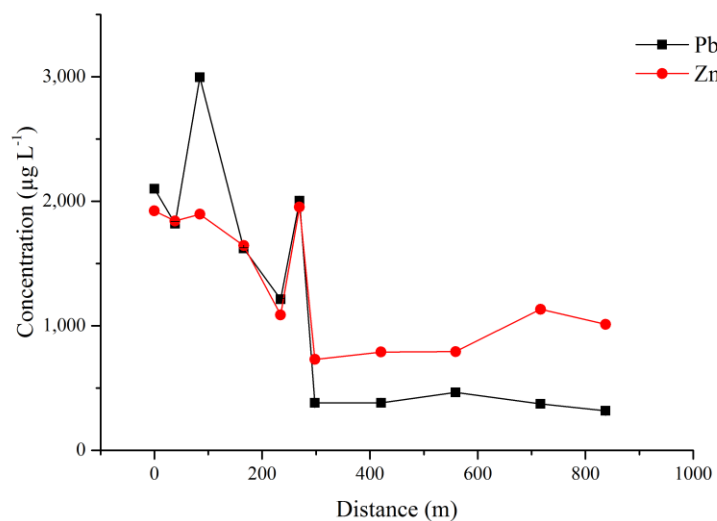


Figure 4-9. Dissolved Pb and Zn in samples collected from the stream leaving the Tyndrum Pb/Zn mine in May 2015.

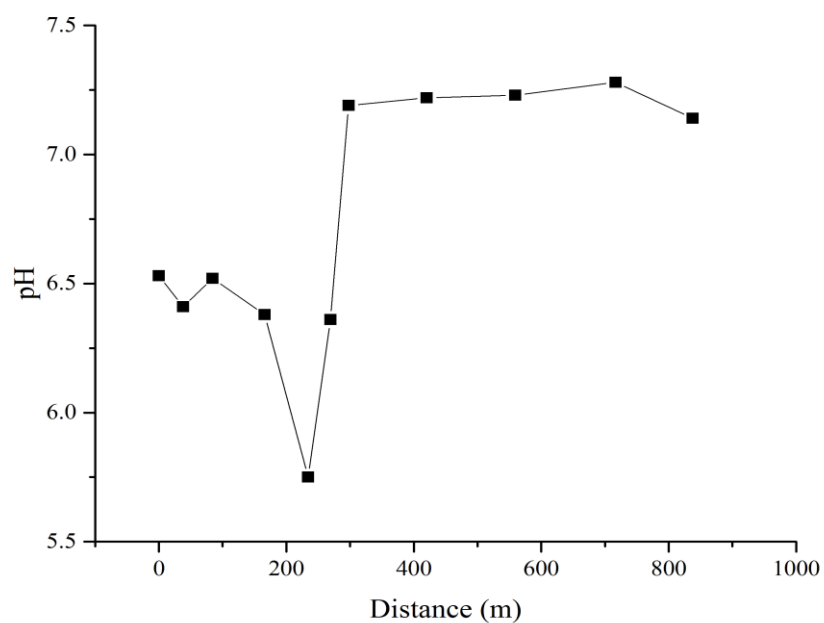


Figure 4-10. Water column pH in samples collected from the stream leaving the Tyndrum Pb/Zn mine in May 2015.

4.5 Tyndrum Discussion

4.5.1 Risk to the Surrounding Environment

The investigation indicated significant PTE loadings in the tailing material, with Pb and Zn of particular concern. Concentrations of total PTE were highly heterogeneous (Figure 4-1-3), with no obvious pattern of distribution or linkage with other site characteristics (in the exception of Pb - K_d and pH), leading to unpredictable concentrations of total PTE across the mine tailings. It is possible that this has been a result of the random dumping of tailing wastes during mining activities, coupled with the different extraction, dressing and processing approaches used under each iteration of active mining on the site (Mills *et al.* 2014), leading to a varied dispersion of PTE enriched material. It may have been assumed that 90 years of abandonment would have allowed natural physical sorting to occur, however the previous evidence of limited aerial dispersion (Mansor 2008), close proximity of the surrounding woodland and limited biological colonisation may have retarded hydrological and aeolian erosive processes to the site and limited physical dispersion within the tailing area.

The transportation of PTE from the site to the wider environment has been previously highlighted to be mainly driven by hydrological dispersion of the tailing material (Farmer *et al.* 1997; MacKenzie and Pulford 2002; Pulford *et al.* 2009) whereas airborne movement of tailing particulates were observed to be minimal (Patrick and Farmer 2006; Mansor 2008). This investigation has provided further evidence of PTE movement from the tailings (Figure 4-9) by demonstrating highly elevated Pb and Zn concentrations in the water column fraction < 45 μm , continuous release of PTE from the abandoned mine and therefore a significant risk to the immediate aquatic environment. Significant decreases in dissolved Pb and Zn and an increase in pH were observed approximately 300 m downstream of the tailings, where a small dam is located, potentially limiting the movement of PTE enriched tailing material. The hydraulic sorting of mine tailings at this point has been described in (Pulford *et al.* 2009) and this investigation supports the evidence that this barrier is continuing to have an important role in the retardation of PTE transportation to

the wider environment. Critically, if the barrier was to fail then there would likely be a substantial loading of PTE into the water column.

Considering the solid phase tailing material, CaCl_2 extractable concentrations of Pb, Cd, and Zn were highly variable and not correlated to total elemental concentrations (Table 4-3) suggesting that elevated concentrations of total PTE in a given area may not necessarily indicate a higher biological risk. Furthermore, K_d coefficients strongly indicate that pH is a significant driver of desorption of Pb into the aqueous phase, underlining potential acidification of the mine tailings as a major risk to greater release of mobile Pb (Table 4-4). For Zn and Cd, the mechanism for controlling desorption is not clear, although due to the correlations of these analogous elements with total S, and the evidence that these elements are generally bound to the organic/sulphide fractions in the tailings (Chapter 6), it would be reasonable to assume that further mobilisation of these PTE is limited unless significant changes in environmental conditions occur.

The findings from XRD, CaCl_2 extractable Pb, Zn, and Cd, and K_d coefficients suggest that it is reasonable to assert that the tailing materials present a significant latent risk to the surrounding water and soil environment. Under the current conditions, the relatively insoluble Pb-carbonates that dominate the tailings produce a minor to moderate exchange from the solid to the aqueous phase, leading to a low, but steady discharge of Pb into the soil solution. These loadings may currently be environmentally tolerable, however with specific reference to Pb, slight decreases in the pH of the tailing matrix would likely promote substantial dissolution of carbonate held Pb, as demonstrated by the strong K_d – pH relationship. The main risk of acidification of the tailings is likely to be the surrounding pine woodland, where the encroachment of acidic soils may promote the mobilisation of Pb.

4.5.2 Key identified Issues for Remediation

The Tyndrum mine tailings present a complex issue in terms of remediation approaches due to the heterogeneity in PTE concentrations, their spatial distribution, speciation and mobility risks. Furthermore, the skeletal tailing

structure and lack of supportive soil constituents (e.g. organic matter, roots) provide barriers to vegetative colonisation and a conventionally functioning soil system.

Considering biologically driven remediation methods, the use of phytoremediation would likely be challenging for several reasons. The tailings offer poor support for plant establishment, as demonstrated by the stressed and deceased trees evident on site that were planted by the Forestry Commission in the 1950s. This leads to the limitation of potential species to those that are both tolerant to nutritional deficits, periodic waterlogging, drought and elevated PTE burdens. Considering total measured tailing concentrations of Pb and Zn at this site, and the current literature on phytoextraction it is unlikely that this technology would be a suitable option. For example, based on the work by (Robinson *et al.* 2015) using a hypothetical system where uniform vegetative growth across the 1.1 ha site was established with an annual 10 tonne biomass production, a mean shoot concentration of 20 mg kg⁻¹, the mean total concentration of Pb for the tailings (~ 12,000 mg kg⁻¹) and a target total concentration of 400 mg kg⁻¹ would take several thousands of years to achieve, assuming that all of the Pb will be available for plant uptake and biomass production is consistent throughout. This method is clearly not viable and so biological removal of Pb from the site is essentially unachievable.

As the primary mode of transportation of PTE from the mine to the wider environment has been attributed to particulate movement (Pulford *et al.* 2009), the use of phytostabilisation as a retention mechanism may provide a suitable solution. In this scenario, plant species would ideally exhibit a dense rooting system and minimise translation of PTE from the root to aerial compartments of the plant to reduce the possibility of PTE transference along the food chain (Raskin and Ensley 1999; Alkorta *et al.* 2010). Furthermore, a consistent and dense ground cover would be preferential to minimise exposed tailing material and reduce the occurrence of surface erosion, with an additional requirement of vegetation cover during the winter months.

To facilitate the growth of vegetation under probable PTE stress, and promote the immobilisation of Pb, organic inputs will likely be required in order to enhance the structure, water retention and nutrient content of the tailing material. Conversely, the addition of organic materials to the tailings may elicit several geochemical changes and either promote or retard PTE mobility depending on alterations to labile organic carbon, pH, soluble elemental inputs such as Fe/Mn/Al, reduction/oxidation processes and biological mechanisms. (Park *et al.* 2011) Therefore, the application of organic amendments must be considered carefully to avoid the possibility of exacerbating PTE mobility. Importantly, the organic amendment to be applied to the tailings would necessitate an increase in pH or provide buffering to acidification to prevent the dissolution of Pb-carbonate materials.

The application of organic materials on their own is unlikely to be a viable option for the Tyndrum tailings as the high annual rainfall and surface erosion processes would likely rapidly remove incorporated materials without a barrier in place. This potential loss of material may then have negative impacts on downstream water quality (Couillard and Li 1993; Ramos *et al.* 2006; Larney *et al.* 2014; Singh *et al.* 2017), exacerbating the pollution profile emanating from the tailings. Therefore, a combined organic input and phytostabilisation approach may be the most useful for the Tyndrum tailings in terms of ecologically driven remedial action.

4.6 Results – Le Bleymard

4.6.1 Concentration and Distribution of PTE in the Mine Tailing Site

Concentrations of total PTE are detailed in (Table 4-6). Similarly to the mine tailings at Tyndrum, total concentrations were highly variable with particularly elevated concentrations of Pb, Cd and Zn compared to EQS and other PTEs such as As, Cr, Co, Cu, Ni and Sb. Calcium chloride extractable PTEs were below detection limits for all measured elements except Pb and Zn, where mean extractable concentrations were 0.02 and 0.008 % of the mean total concentrations respectively.

Table 4-6. Mean total and CaCl₂ extractable concentrations of selected PTE at the Le Bleymard Pb/Zn mine tailings. n = 24. LOD = limit of detection. Elevated concentrations of PTE highlighted in bold. *Environmental Quality Standards are based on soil concentrations for land deemed for industrial use (CCME 2018).

Element	Total Concentration (mg kg ⁻¹) (± std. dev)	Environmental Quality Standard* (mg kg ⁻¹)	CaCl ₂ Concentration (mg kg ⁻¹) (± std. dev)
As	68.6 (± 44)	12	< LOD
Cd	166 (± 89)	22	< LOD
Co	1.56 (± 2.3)	300	< LOD
Cr	37.2 (± 26.4)	87	< LOD
Cu	55.0 (± 48.3)	91	< LOD
Ni	29.1 (± 52.7)	89	< LOD
Pb	6,593 (± 5,501)	600	1.6 (± 0.33)
Sb	27.4 (± 28.1)	40	< LOD
Zn	42,448 (± 16,250)	410	3.4 (± 5.1)

Concentrations of total Pb at the tailings were found to increase in a westerly downhill direction, with the highest concentrations found at the bottom of the tailing system (Figure 4-11). The highest measured point of total Pb was located at the south west of the tailing area, close to the farm buildings. Total Pb was strongly inversely related with median particle size (Figure 4-12) which decreased in a similar direction to increasing total Pb concentrations (Figure 4-13).

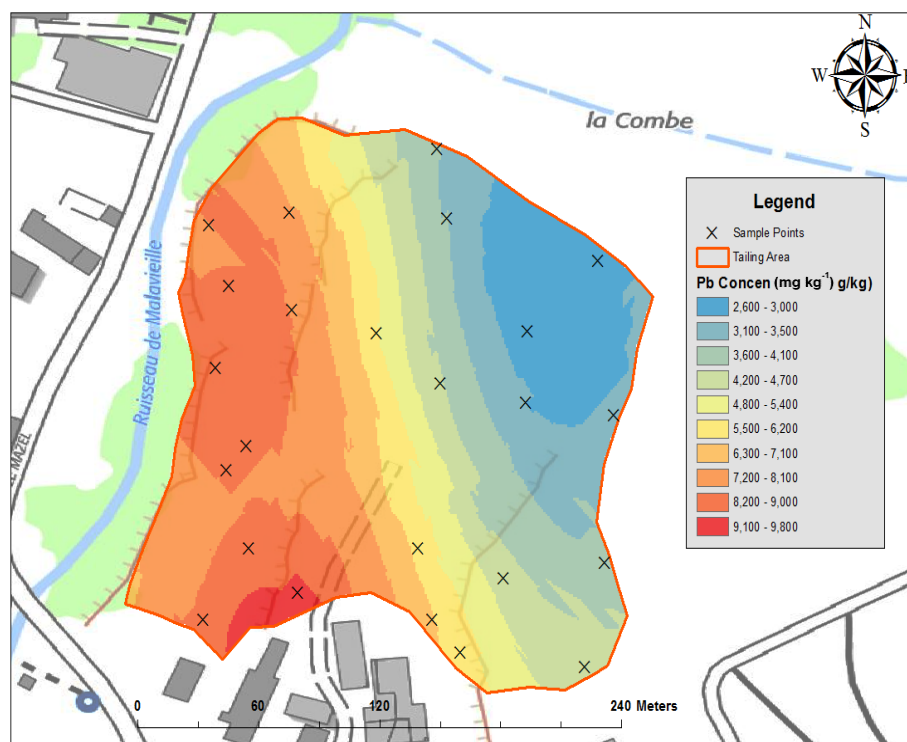


Figure 4-11. Empirical Bayesian Kriging of total Pb concentrations at the Le Bleymard Pb/Zn mine.

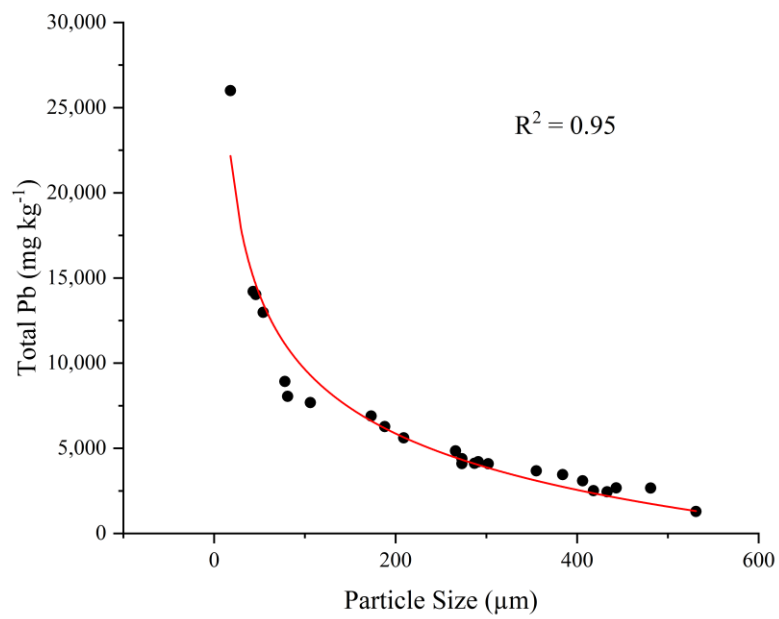


Figure 4-12. Correlation between median particle size and total Pb concentration at the Le Bleymard Pb/Zn mine.

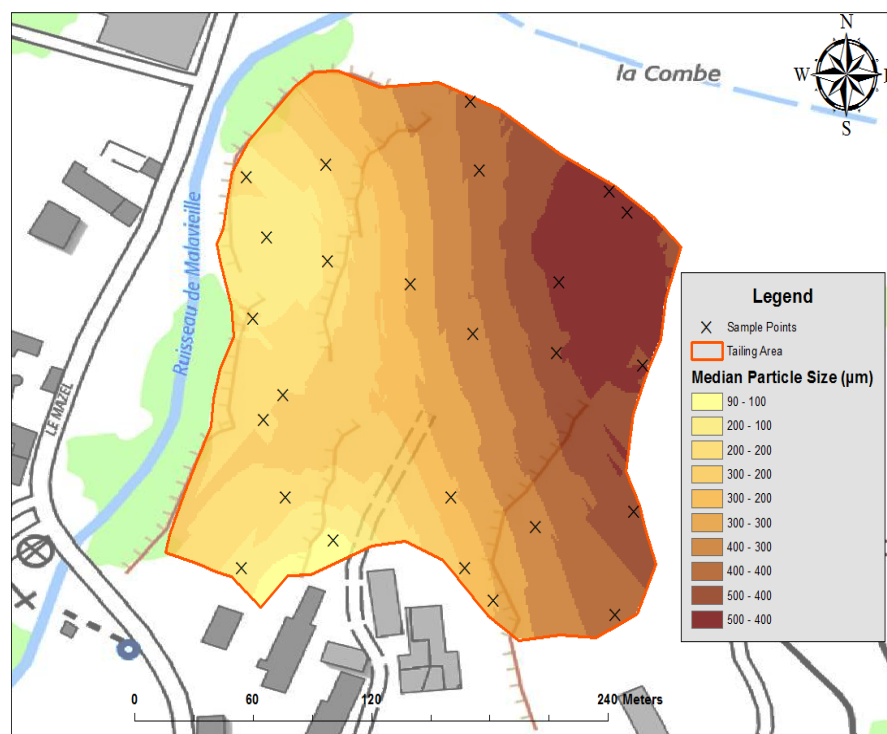


Figure 4-13. Empirical Bayesian Kriging of median particle size at the Le Bleymard Pb/Zn mine.

Total Zn concentrations were observed to be highest in the bottom western section of the tailings, with some evidence of elevated concentrations in the upper north-eastern area (Figure 4-14). Lower concentrations were observed in the central area of the tailings. Total Zn was not correlated with median particle size and was weakly correlated with total Cd ($R = 0.52$). Similarly to Zn, elevated total Cd concentrations were found in the lower and the north-eastern areas of the tailings, with a lower concentration gradient in the centre. Conversely, the highest concentrations of Cd were observed in the south-western region of the tailings (Figure 4-15).

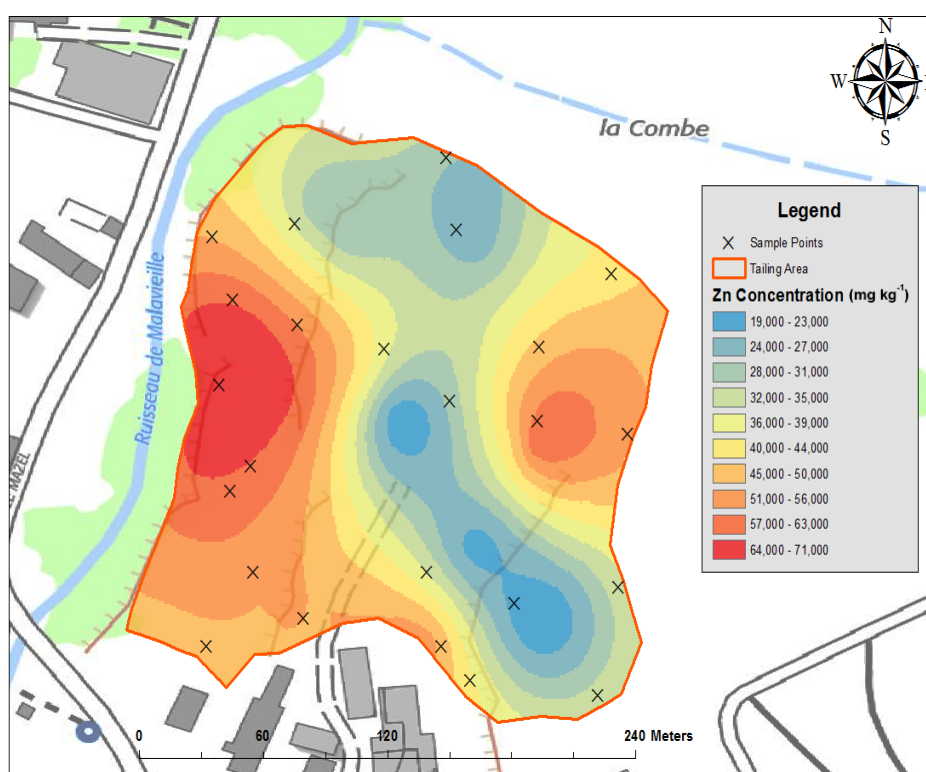


Figure 4-14. Empirical Bayesian Kriging of total Zn concentration at the Le Bleymard Pb/Zn mine.

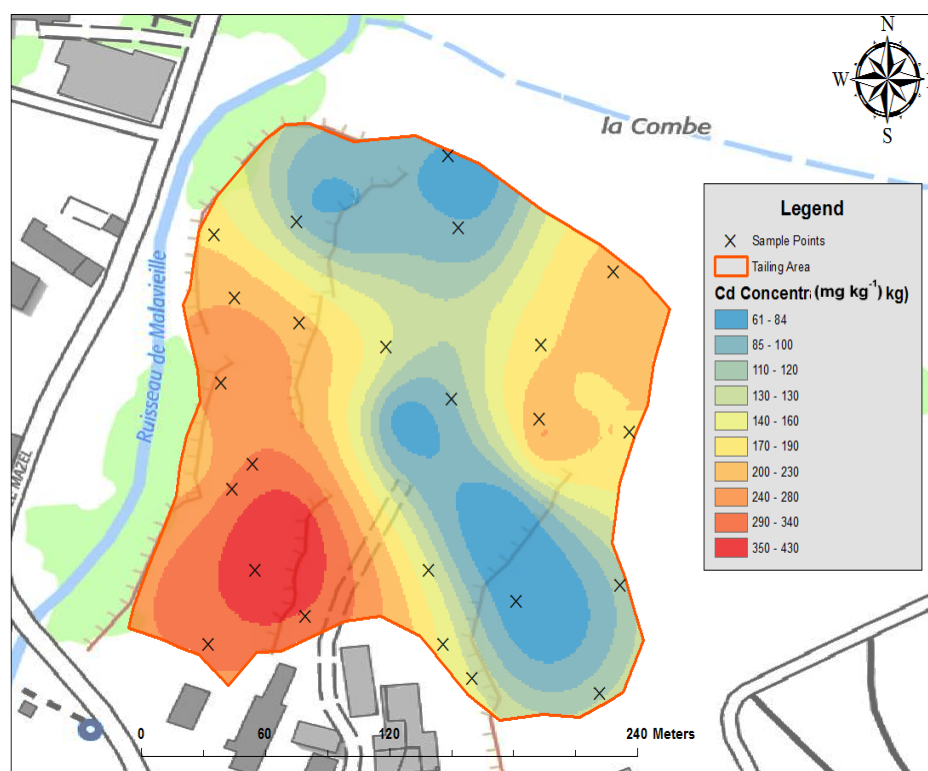


Figure 4-15. Empirical Bayesian Kriging of total Cd concentration at the Le Bleymard Pb/Zn mine.

4.6.2 Partition Coefficients for PTE in the Le Bleymard Mine Tailings.

Partition coefficients for Pb Zn and Cd were high, indicating a low potential for desorption of PTE into the aqueous phase under current conditions (Table 4-7). Partition coefficients were not influenced by pH differences in the site or by other measured characteristics.

Table 4-7. Partition Coefficients for Pb, Zn and Cd at the Le Bleymard Pb/Zn tailings. Note: Cd coefficients were not able to be calculated due to CaCl_2 extractions determined as < LOD.

Element	LogK _d Coefficient	Min	Max
Pb	4.5	4.2	4.8
Zn	5.2	4.2	5.6
Cd	-	-	-

4.6.3 Concentration and Distribution of Tailing Fertility in the Mine Tailing Site

Measured tailing parameters are detailed in Table 4-8. The mine tailings at Le Bleymard show some similarities to that of the Tyndrum site, with low TN/TC,

organic matter content and a range in median particle size. The tailing pH is slightly neutral with high concentrations of basic cations (Ca and Mg).

Table 4-8. Values of selected tailing parameters at the Le Bleymard lead mine tailing. n = 24

Parameter	Value (\pm std. dev)
pH	7.2 (\pm 0.3)
Total N (%)	0.04 (\pm 0.1)
Total C (%)	8.3 (\pm 1.6)
Loss on ignition (%)	6.1 (\pm 2.7)
Total Ca (mg kg ⁻¹)	83,399 (\pm 19,989)
Total P (mg kg ⁻¹)	1,015 (\pm 593)
Total Fe (mg kg ⁻¹)	12,664 (\pm 7,928)
Total Mn (mg kg ⁻¹)	831 (\pm 697)
Total Mg (mg kg ⁻¹)	27,015 (\pm 16,242)
Total S (mg kg ⁻¹)	4,484 (\pm 2,168)
Median Particle size range (μ m)	18 - 531

The distribution of TC was weakly spatially aligned with vegetation presence and generally highest in the lower north-western sections of the tailing system (Figure 4-16). In contrast, TN was mainly concentrated in the northern section of the tailing system, with some elevated presence in the south eastern and south western areas (Figure 4-17). Total N was not spatially linked with vegetation or correlated with TC.

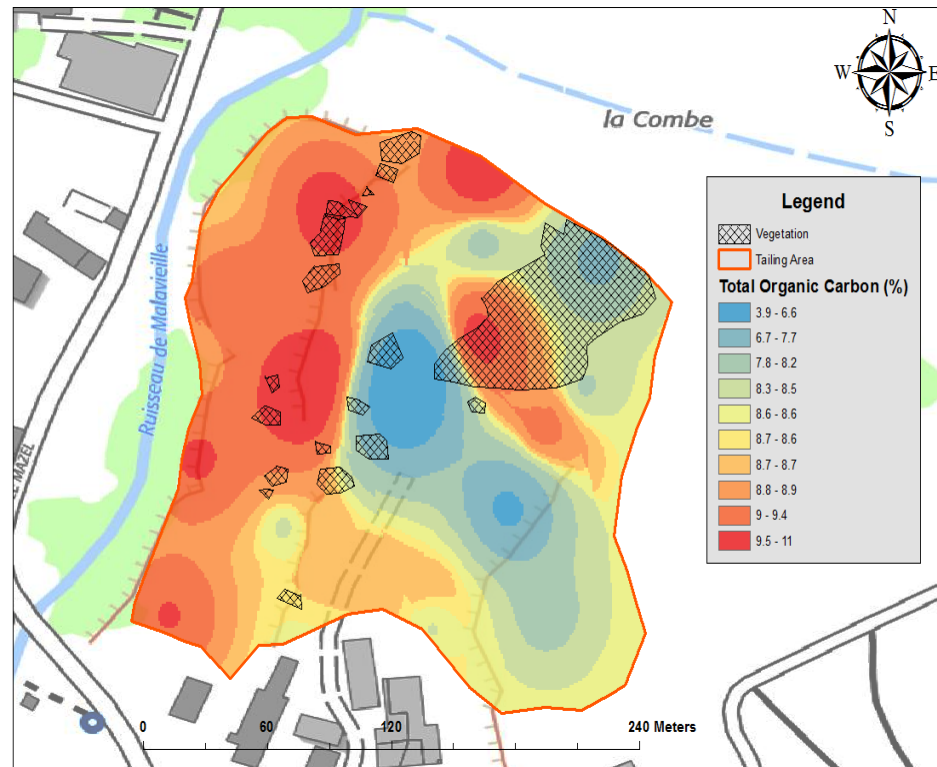


Figure 4-16. Empirical Bayesian Kriging of total organic carbon at the Le Bleymard Pb/Zn mine.

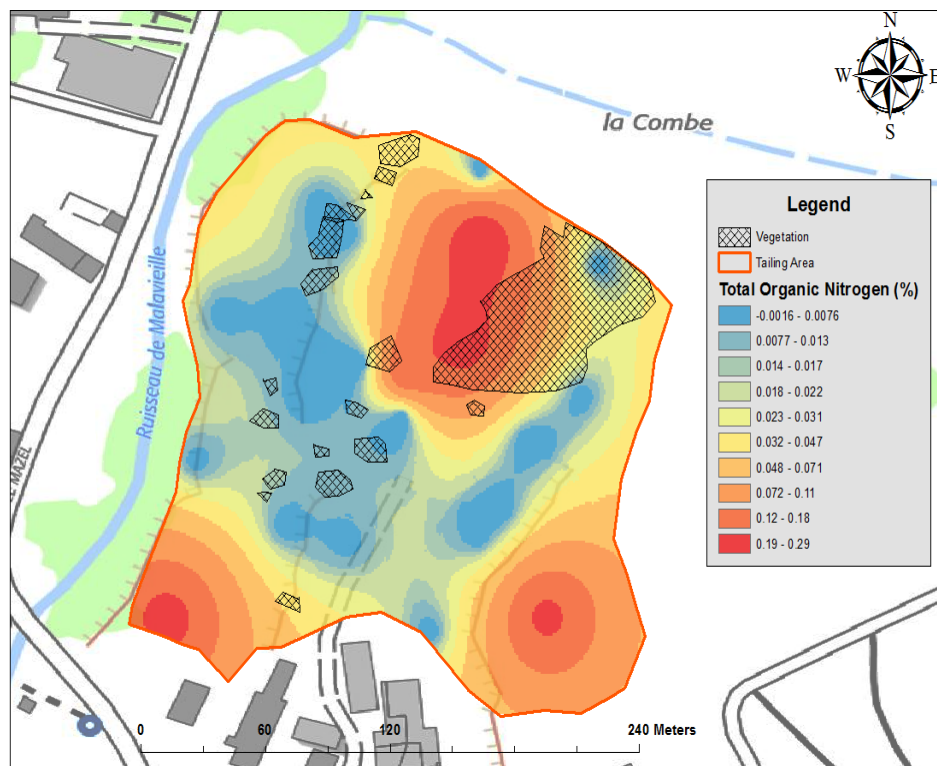


Figure 4-17. Empirical Bayesian Kriging of total organic nitrogen at the Le Bleymard Pb/Zn mine.

The pH of the tailings was uniformly distributed, with a consistent value of 7.2 (± 0.3) across the tailing area.

X-ray diffraction of the Le Bleymard tailings exhibited a carbonate dominated geological basis, with significant presence of dolomite ($\text{CaMg}(\text{CO}_3)_2$), calcite (CaCO_3), smithsonite and minrecordite ($\text{CaZn}(\text{CO}_3)_2$) (Figure 4-18). The presence of galena was also recorded.

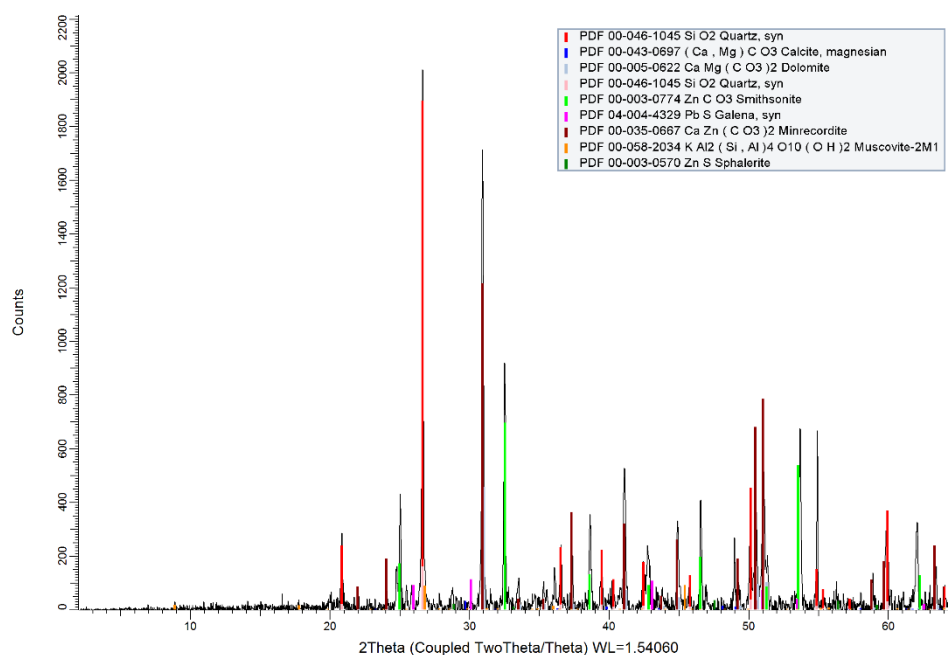


Figure 4-18. X-ray diffraction of a typical sample sourced from the Le Bleymard Pb/Zn mine tailings.

4.6.4 Plant Species on the Le Bleymard Mine

A diverse range of plant species were identified on the tailings, with a common grouping of upland, calcareous species dominating the vegetation range. Vegetation cover was irregular throughout the tailing system, with the majority of plant cover located in the higher north-eastern region of the mine. The most common species identified on the site were *N.caerulescens*, matgrass (*Nardus stricta*) and coltsfoot (*Tussilago farfara*).

4.6.5 Waterbody Concentrations of Cd, Pb and Zn

Dissolved Pb concentrations in the Ruisseau de Malavieille at Le Bleymard generally increased as the stream passed the mine tailings, with a peak in concentration at approximately 250 m (Figure 4-19). Concentrations of Pb from

this distance differed with sampling date, with those collected in April 2017 exhibiting a decreasing trend with distance and samples from April 2016 demonstrating a consistent concentration until approximately 3 km. Samples collected in October 2017 showed a significant decrease in concentration compared to other sampling periods ($p = < 0.001$) and thereafter a general increase in concentration with distance (Figure 4-19).

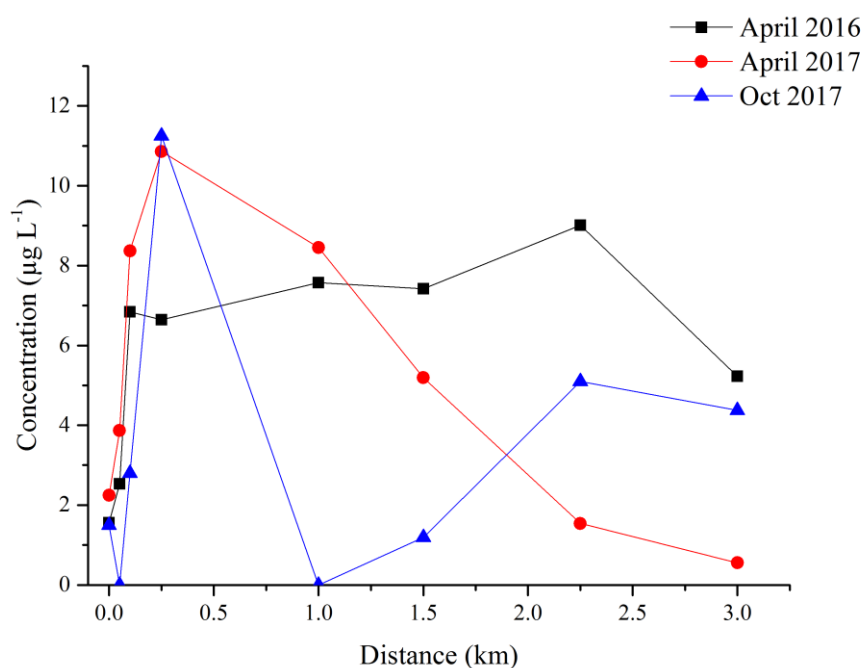


Figure 4-19. Dissolved Pb in the water column between April 2016 - October 2017.

Concentrations of dissolved Cd in the Ruisseau de Malavieille generally increased as the waterbody flowed past the tailings in a similar manner to Pb (Figure 4-20). Concentrations of Cd in April 2017 were significantly higher than those in April 2016 and October 2017 ($p = < 0.001$) until approximately 25 km downstream, where concentrations were significantly lower thereafter ($p = < 0.001$). Concentrations in April 2016 showed a general increase with distance, with October 2017 samples exhibiting a similar trend.

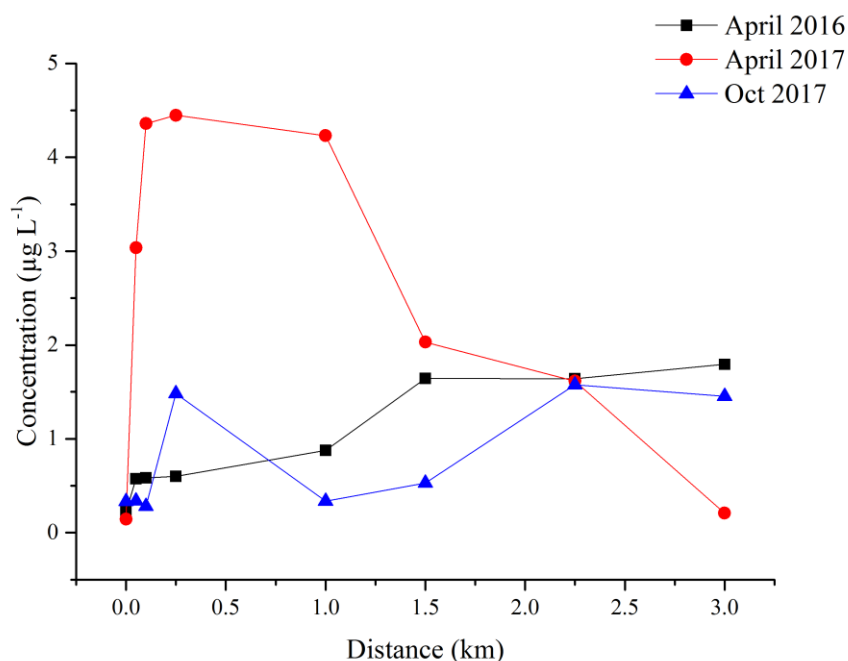


Figure 4-20. Dissolved Cd in the water column between April 2016 - October 2017.

Similarly to Pb and Cd, Zn concentrations increased with proximity to the tailings with samples collected in April 2016 and October 2017 exhibiting the highest concentrations of Zn at approximately 300 m downstream ($p < 0.001$) (Figure 4-21). The concentration of Zn in the April 2017 samples decreased steadily with distance whereas the samples collected in October 2017 demonstrated a rapid decline between ~ 300 m – 1.0 km, thereafter increasing in concentration with distance from the mine. The samples collected in April 2016 suggested a continuous increase in Zn concentration as the watercourse passed the tailing site and increased until approximately 2.25 km whereby Zn declined in the water column.

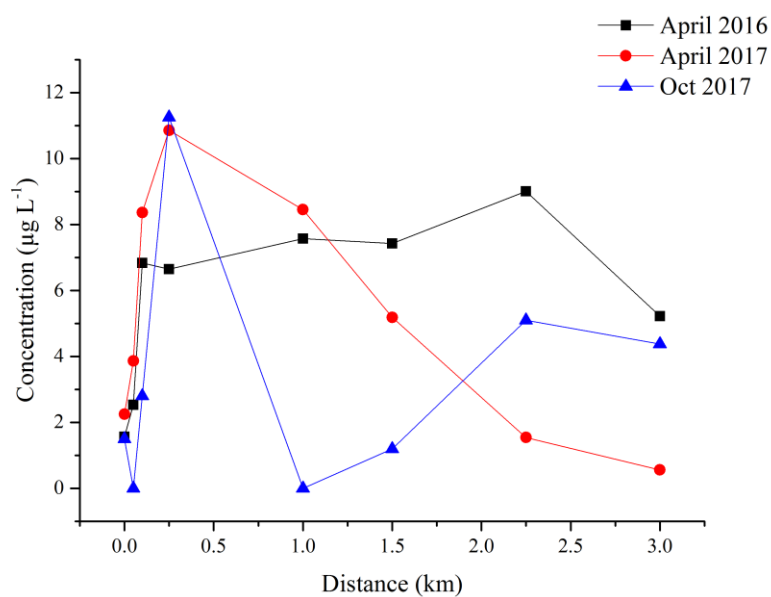


Figure 4-21. Dissolved Zn in the water column between April 2016 - October 2017.

4.6.6 Waterbody pH

The pH of the Ruisseau de Malavieille increased with distance from the mine, with an initial pH of $6.5 (\pm 0.1)$ rising to $7.3 (\pm 0.06)$ at 3 km ($p = < 0.001$) (Figure 4-22). No significant differences were found in pH between sampling dates across the distance sampled ($p = > 0.05$).

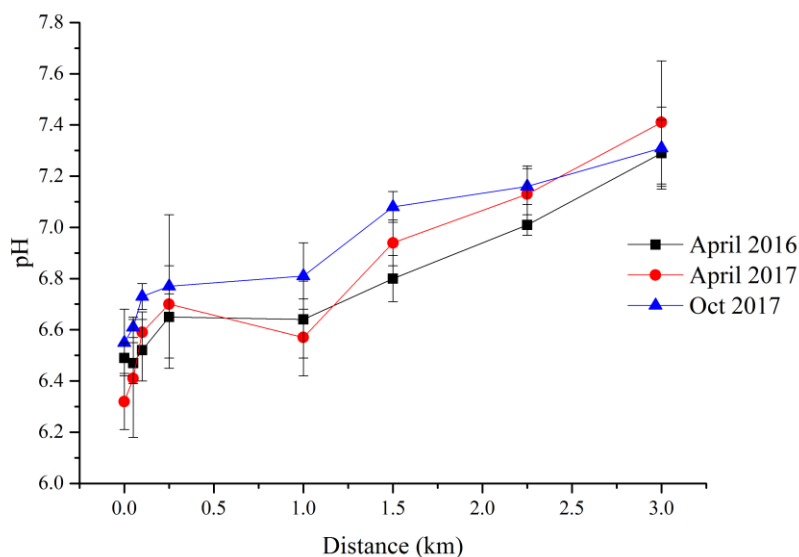


Figure 4-22. Water column pH in samples collected from the Ruisseau de Malavieille between April 2016 - October 2017.

4.7 Discussion

4.7.1 Risk to the Surrounding Environment

This study suggests that there are significant concentrations of Pb, Zn and Cd at the Le Bleynard tailings, with potential to introduce a substantial risk to the surrounding environment and local population. Empirical Bayesian Kriging interpolation suggested that the highest concentrations of Pb were found at the base of the tailing mounds and strongly inversely correlated to particle size. This implies that Pb is mainly associated with the clay fraction of the tailing matrix and therefore highlighting the risk for increased transportation either as dust or suspended material in a water column (Aghili *et al.* 2018; Gil-Loaiza *et al.* 2018). Furthermore, the westerly and downhill trends of both total Pb and median particle size suggests that there is a migratory effect of total Pb in the tailing area and an accumulation of Pb in the lower regions of the mine. The evidence of several small gullies leading from the mine directly into the watercourse in the regions with elevated Pb concentrations suggests a substantial input of Pb enriched material into the Ruisseau de Malavieille. A similar conclusion could be drawn for total Zn and total Cd based on the evidence for higher concentrations of these elements on the western side of the tailings, however, the dispersion mechanism for these elements may be controlled by a different variable, as no evidence for either element and particle size was observed.

Measured dissolved concentrations of Pb, Cd and Zn in the Ruisseau de Malavieille suggest that the tailings present a point source of pollution for the waterbody, with significant increases of all assessed PTE as the stream passes the tailings. Despite the high total loadings of PTE from the material, dissolved concentrations of Pb, Cd and Zn are surprisingly low, indicating a high retention of PTE to the solid phase. The concentrations and trends of these elements appear to vary substantially over small time differences, potentially suggesting a highly seasonal variation in PTE loading in the water column. Reasons for this could include variations in sediment input to the stream during periods of heavy rainfall or extensive dry periods reducing the stream volume. The elevation of

pH may also be attributable to the input of the carbonate rich tailing material (increasing stream pH as it passes the tailing site). However, based on the lack of relationship between pH and K_d there is no evidence that pH is a driver when considering periodic differences in PTE concentrations, and there may be another mechanism behind the low desorption rates and presence of dissolved PTE in the water column, such as precipitation of PTE or adsorption to tailing surfaces (Elkhatib *et al.* 1992; Appel and Ma 2002; Alloway 2012; Jalali and Moradi 2013).

The K_d coefficients suggest that the potential for desorption of Cd, Pb and Zn are very low under current environmental conditions, likely due to the near neutral/slightly alkaline pH and the strong electrostatic attraction of these elements to the solid phase (Lafuente *et al.* 2008; Jalali and Moradi 2013). This strong attraction is probably a consequence of the carbonate rich, geologic origin of the tailing material. This limited desorption capacity explains the low water column concentrations of PTE despite evidence from high particulate loading and runoff into the Ruisseau de Malavieille. Therefore, it can be considered that under the current conditions the tailings present a low risk to the immediate soil and aquatic environment due to the high buffering capacity.

In contrast to the low chemical mobility of the tailing area there is a significant risk to airborne dispersal. A study by Ploquin, (2010) suggested tailing material disturbance led to an increase in the blood Pb concentrations of local residents in Le Bleymard. The arid conditions in the region exacerbate this risk as tailing dust is regularly blown from the mine around the surrounding environment. Therefore, inhalation of fine particulates with absorbed Pb concentrations could have significant risks to human and livestock health. Furthermore, the aerial dispersal of the tailing material could introduce PTE enriched particulates into areas of differing environmental conditions, (such as surrounding arable land of lower pH), leading to a possibly substantial release of bioavailable PTE.

Overall, it is expected that the current environmental regime at Le Bleymard generates a low risk of PTE mobilisation and bioavailability within the immediate area. However, the association with small particulates and elevated

Pb give rise to concerns of the ready transportation of PTE enriched material to both downstream and downwind receptors.

4.7.2 Key Identified Issues for Remediation

Similarly to the Pb/Zn tailings at Tyndrum, the site at Le Bleymard poses several difficulties in terms of remediation approaches. Similar discussions from section 4.5.2 are also relevant here regarding the use of phytostabilisation as opposed to phytoextraction, and the risks of applying organic amendments.

Specifically, the carbonate rich tailings at Le Bleymard show evidence of a high PTE retention, however, upon acidification a substantial release of Pb, Zn and Cd will occur (Appendix 1), provoking concern over environmental changes that may transpire during remedial efforts. Unlike the encroaching woodlands in Tyndrum it is doubtful that the immediate surrounding arable landscape would lead to a substantial increase in acidic conditions, and so desorption risks would likely only occur from material that has been transported away from the tailing area or by inputs of materials (e.g. atmospheric deposition of acidifying pollutants) that would promote this reaction.

The application of acidic, chelating or materials rich in soluble organic carbon may elicit a substantial increase in mobility of PTEs from the tailings into the aqueous phase (Alloway and Jackson 1991; Ashworth and Alloway 2008), however the availability of essential plant nutrients are often diminished at an elevated soil pH, and so a lower pH would potentially be more beneficial for vegetative colonisation (Hopkins and Ellsworth 2005; Cross and Lambers 2017). Moreover, the presence of small, PTE enriched particulates observed to be adjacent to the Ruisseau de Malavieille exacerbate this risk, with a potentially greater environmental consequence if mobility of PTE is increased in these areas.

The identified plant species at the mine generally did not provide substantial rooting or surface cover, limiting the applicability of promoting the use of these species that are found on the tailings. However, the vegetative diversity in the Lozère region is high and so a suitable local phytostabilisation species may be

able to be applied with no risk of introducing non-native plant material to the site. An important consideration is the effect that vegetation may have on PTE mobility. Plants have been well documented to directly promote the mobilisation of nutrients and elements in the rhizosphere and introduce chelating compounds in the form of low molecular weight organic acids under conditions of nutrient stress (Mench and Martin 1991; Jones 1998; Römheld and Awad 2000; Dakora and Phillips 2002). Therefore, a phytoremediation approach may have unintended mobilisation effects on the tailing material and may promote desorption of PTE from the previously stable solid phase. It is possible that this process may locally mobilise PTE in the tailing system, increasing PTE transportation and magnify the PTE burden on vegetative systems themselves by increasing the bioavailability of Pb, Cd and Zn, and therefore warrants investigation prior to implementation on site.

Similarly to the Tyndrum site, the retention of tailing material, and in particular the < 100µm fraction would be a priority for limiting Pb transportation from the mine to the wider environment and indeed to reduce the movement within the mine itself. Remedial efforts could be considered in two ways; directly in the lower regions of fine tailing material to reduce the movement of these fine sediments, or in the higher, eastern side of the mine to limit movement and accumulation of PTE in the lower regions. Ideally an implementation of both systems would hopefully reduce the issue of PTE transportation.

4.8 Site Comparisons

The tailings at Tyndrum and Le Bleymard have both parallel and distinct characteristics. The sites share similarities in terms of elevated and heterogeneous distributions of PTE, alongside low concentrations of organic matter, TN and TC. This presents a challenging landscape in terms of ecologically driven remediation as both sites show evidence of a hostile environment for vegetation colonisation, aside from specialist species found on the tailing heaps. Furthermore, both tailing areas have demonstrated similar environmental transportation routes, mainly in the form of hydrological erosion and particulate loss into adjacent waterbodies. Moreover, there are comparable

risks from movement of PTE from the solid to the aqueous phase if environmental change resulted in acidification of the tailings and dissolution of carbonate-held and weakly-associated PTE. Overall, there is evidence that remedial actions require the retardation or retention of PTE enriched material from erosive pressures to prevent the movement of the tailings into the wider environment. Importantly, remediation should also aim to either reduce the risk of dissolution or desorption processes or immobilise PTE by promoting their incorporation into more stable chemical species.

In contrast there are significant differences between the two abandoned tailings. The site at Tyndrum has significantly lower K_d coefficients for Pb and Zn ($p < 0.001$), demonstrating that the potential for PTE mobility is much higher than that of Le Bleymard, evidence of which is seen in the water column of the Ruisseau de Malavieille which is orders of magnitude less than that of the stream flowing from the Tyndrum tailings (Table 4-9).

Table 4-9. Comparison of Log K_d coefficients for Tyndrum and Le Bleymard mine tailings.

Element	Tyndrum	Le Bleymard
Pb	2.9	4.5
Zn	2.4	5.2
Cd	2.0	-

Conversely, the aerial dispersion risks from Tyndrum are low in comparison to Le Bleymard, where dust creation has led to elevated blood Pb concentrations in local residents, likely due to the climate, lack of plant cover and the aridity of the tailings. Further differences are seen in terms of geology, the movement of particulates, physical sorting processes and overall PTE concentrations as discussed earlier in this chapter. It could be argued that the mine tailings at Le Bleymard exhibit a lower present-day risk to PTE pollution than Tyndrum due to the relatively static environment, high buffering capacity and greater retention of PTE. However due to the generally higher total concentrations of PTE observed at the tailings, acidification or desorption processes could elicit a significant mobilisation of PTE coupled with a higher inhalation risk than found

at the Tyndrum tailings. It is therefore clear that approaches to devising potential remediation systems for these sites in this thesis will be comparable in some respects; for vegetation to be established an improvement in tailing nutrient content and potentially some form of protection from labile PTE would be required for both sites. However, different plant species and considerations of appropriate organic materials will be required to avoid the possibility of aggravating PTE mobility rather than reducing it.

A summary describing the findings of this chapter are given in (Table 4-10) below.

Table 4-10. Summary table of key findings at Tyndrum and Le Bleymard in Chapter 4.

Parameter	Tyndrum	Le Bleymard
pH	4.3 – 7.4 Slightly acidic	6.5 – 8.0 Neutral to alkaline
Total PTE (mg kg⁻¹)	Pb: 500 - 40,000 Zn: 130 – 17,000 Cd: <LOD - 70	Pb: 1,300 - 26,000 Zn: 19,000 – 69,000 Cd: 60 - 430
PTE Mobility (Log K_d)	Pb: 1.9 – 3.9 Zn: 1.7 – 3.9 Cd: 1.4 – 2.9	Pb: 4.2 – 4.8 Zn: 4.2 – 5.6 Cd: No data
Distribution of PTE	Highly heterogeneous with no identified transport mechanism	Migration of PTE from upper to lower areas, Pb associated with small particulates
PTE Pathways	Hydrological erosion from small streams and into surrounding watercourses	Hydrological erosion and deposition into watercourses. Dust and inhalation
Available Plant Nutrients	Low, correlated with plant growth and areas of vegetation	Low, correlated with plant growth and areas of vegetation
Vegetation	Acidic grassland species, heather species and some impacted trees	Calcareous adapted species and grasses, hyperaccumulator species
Geological Baseline	Weathered galena and carbonate held Pb minerals	Dolomitic and calcareous, high presence of smithsonite
Remedial Issues	Low nutrient content, high availability of PTE to plants, risk of acidification	Potentially low bioavailability of plant nutrients, high acidification risk

Chapter 5. Phytotoxicity Investigation

5.1 Introduction

This chapter investigates the phytostabilisation potential of plant species naturally colonising the tailing heaps at Tyndrum and Le Bleymard. The chapter is split into two main components: an initial screening survey to identify species that exhibit resilience in terms of root biomass and surface cover when exposed to elevated soil PTE and poor nutrient conditions, and a secondary survey to assess the species identified in phase 1 in terms of PTE accumulation and compartmentalisation in the aerial and subterranean sections of the plant.

5.2 Methodology

5.2.1 Phase 1 Methodology: Plant Tolerance to Tailings

Ten grams of the relevant seeds of the selected plant species were evenly sown into pots containing a synthesised mine tailing representing the chemical and physical environment of Tyndrum and Le Bleymard. The plants were grown in a glasshouse in a totally randomised structure (generated by Minitab v.16) for 90 days before calculating the percentage of vegetative surface cover of each replication. Subsequently, the entire plant was harvested, separating the roots and shoots and determining the dry weight biomass. Each species was assessed for biomass generation and the information used to inform the second phase of the experiment (section 5.4.2). A full description of the methodology for phase 1 can be found in Chapter 3, section 3.3.6.

5.2.2 Phase 2 Methodology: Uptake and Translocation

From the initial survey, two plant species were selected; *A.capillaris* and *F.ovina*, to be brought forward to a more detailed study regarding PTE uptake and translocation. Seeds of *A.capillaris*, *F.ovina* and a 1:1 mixed ratio of both species were sown into 500 ml plastic pots containing GWC spiked with 800 mg kg⁻¹ of Pb and Zn and grown in a totally randomised structure for 60 days in a glasshouse.

After this period the plants were harvested, separated into the roots and shoots, and assessed for total N content (shoots only), dry weight biomass and elemental

concentrations. The GWC was assessed for CaCl_2 and total Pb and Zn concentrations. Several metrics including translocation factors, bioaccumulation coefficients and extraction efficiencies were determined for the investigated species (Chapter 3, section 3.8). A full description of the methodology can be found in Chapter 3, section 3.3.7.

5.2.3 Selection of Plant Species

From the vegetation survey detailed in Chapter 1, several species were identified as growing on the tailings at Tyndrum and Le Bleymard. From these results the most commonly identified species, alongside some of those which have been noted in the wider literature as tolerating high concentrations of Pb and Zn found in the local region were selected for the initial screening process.

In addition to these species, which were either found extensively on site, or were regionally relevant were also included to expand the scientific knowledge on species that may be suitable for phytoremedial purposes (Table 5-1). A review of the species to be used in this chapter can be found in Chapter 3, section 3.3.5.

Table 5-1. Native presence of phase 1 tested species found at Tyndrum or Le Bleymard.

Species	France	UK
<i>C. bursa-pastoris</i>	✓	✓
<i>M. caerulea</i>	✓	✗
<i>C. hirsuta</i>	✓	✗
<i>N. caerulea</i>	✗	✓
<i>A. capillaris</i>	✓	✓
<i>A. vinealis</i>	✓	✓
<i>L. angustifolia</i>	✗	✗
<i>F. Ovina</i>	✓	✓

5.2.4 Data Presentation and Statistical Analysis

Graphical and statistical outputs were calculated using a combination of Origin 2016 and Minitab v.18. Statistical differences were determined using One-Way

ANOVA and Tukey's family grouping at a significant level of $P < 0.05$. Data were log-transformed to achieve normality where necessary.

Data relating to the CaCl_2 extractable and total Pb/Zn concentrations of the spiked synthesised tailings and GWC can be found in Appendix 12

5.3 Results – Phase 1: Biomass Partitioning

5.3.1 Shoot and Root Biomass Partitioning

Root and shoot biomasses of the selected species are given in Figure 5-1. From the group representing the Le Bleyard tailing material (Figure 5-1 A), *C.pastoris* and *N.caerulescens* were found to exhibit significantly higher shoot biomass ($p = < 0.001$) compared to *A.capillaris*, *A.vinealis*, and *F.ovina*. No growth or seedling emergence was observed in *L.angustifolia*, upon inspection of the samples.

For root biomass, *C.pastoris* and *N.caerulescens* exhibited significantly lower biomass ($p = < 0.001$) than *A.capillaris*, *A.vinealis*, and *F.ovina*. Root or shoot biomasses were not significantly different between spiked or control groups for each respective species ($p = > 0.05$).

Shoot biomass for the Tyndrum group (Figure 5-1B) was significantly greater in *C.pastoris* and *C.hirsuta* compared to *A.capillaris*, *A.vinealis*, *M.caerulea* and *F.ovina* ($p = < 0.001$). No significant differences in shoot biomass were found between other species or between spiked and control groups within species ($p = > 0.05$).

Root biomass in the non-spiked *M.caerulea* group was significantly greater than both spiked and control groups of *F.ovina*, *C.pastoris*, *C.hirsuta* and the spiked group of *A.vinealis* ($p = < 0.001$). Both the spiked and control groups of *C.hirsuta* had significantly lower root biomass than the *A.capillaris*, *F.ovina*, and *M.caerulea* treatments ($p = < 0.05$). The root biomass of the spiked treatment of *A.vinealis* was significantly lower than the control of the same species ($p = < 0.001$).

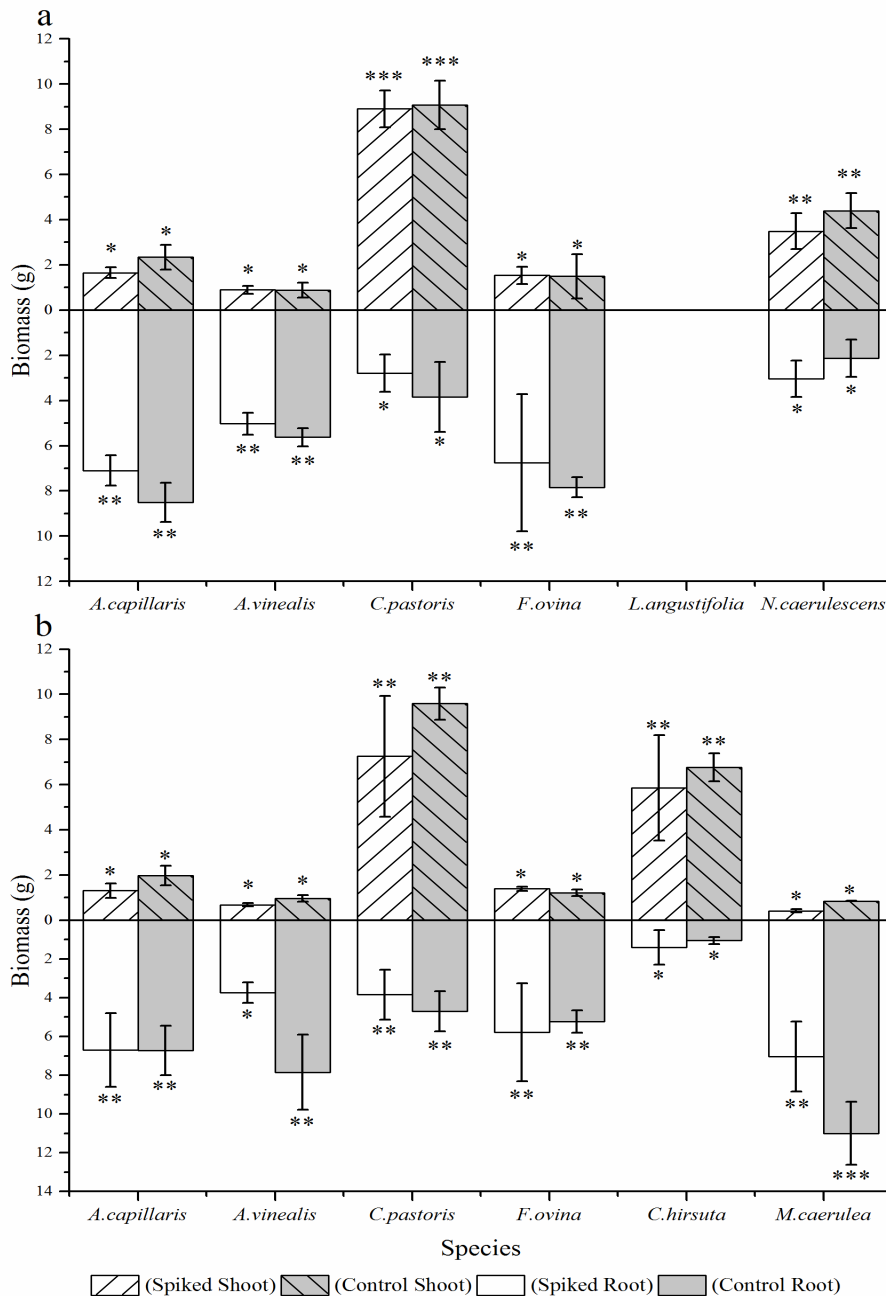


Figure 5-1. Shoot and root biomass of phase 1 spiked and control plant species in A) Le Bleymard and B) Tyndrum synthetic tailings. * indicate significant differences at $\alpha = 0.05$.

5.3.2 Surface Cover

The percentage cover of spiked and control groups in the synthesised Tyndrum tailings is displayed in Figure 5-2. A lower surface cover was found in both spiked and control *C.pastoris* and *C.hirsuta* groups, as well as the spiked *M.caerulea* treatment, as compared to both groups of *A.capillaris* and *F.ovina*

($p = < 0.001$). No other significant differences were found between groups, with the exception of a lower surface cover found in the spiked *M.caerulea* treatment compared to the control ($p = < 0.05$).

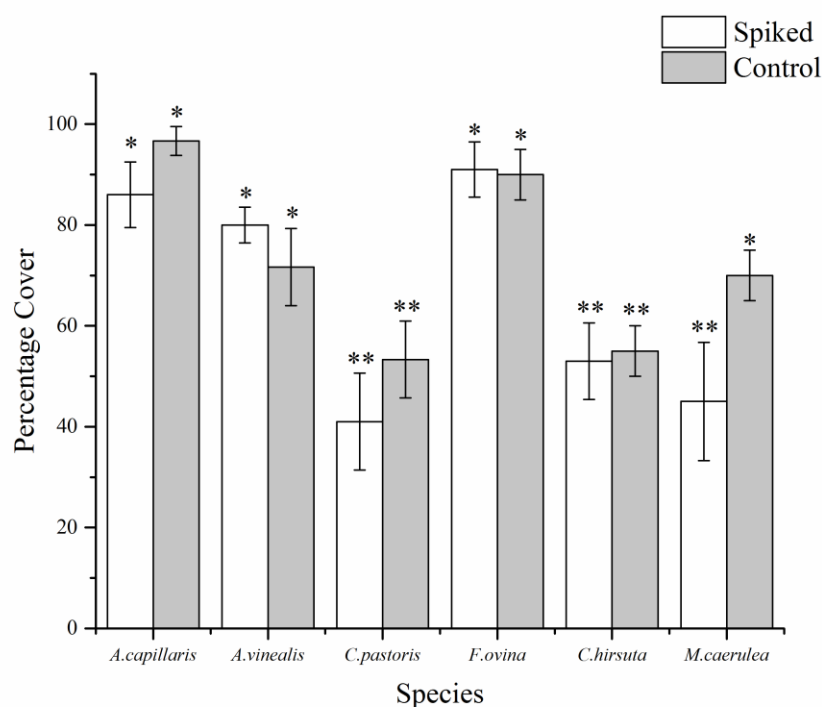


Figure 5-2. Surface percentage cover of the phase 1 phytotoxicity experiment in the synthesised Tyndrum tailings.

In the synthesised Le Bleymard tailings *C.pastoris* and *N.caerulescens* exhibited significantly lower percentage cover compared to *A.capillaris*, *A.vinealis*, and *F.ovina* ($p = < 0.001$) (Figure 5-3). Additionally, both groups of *F.ovina* were found to demonstrate significantly higher surface cover than the spiked *A.vinealis* group. No other significant differences were observed between species or groups ($p = > 0.05$). As previously mentioned, no development was observed in *L.angustifolia*.

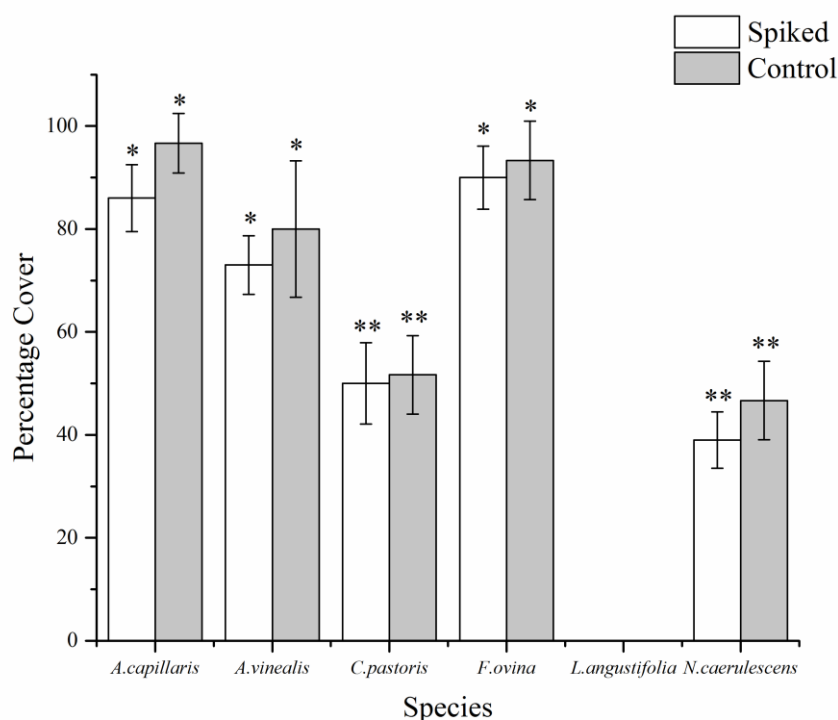


Figure 5-3. Surface percentage cover of the phase 1 phytotoxicity experiment in the synthesised Le Bleymard tailings.

5.3.3 Discussion: Phase 1

From the initial screening survey it is clear that several of the species tested would not be suitable as tools for phytostabilisation on either tailing site.

In the synthesised Le Bleymard samples *L.angustifolia* did not demonstrate growth in either the control or spiked groups, suggesting that the poor chemical and physical conditions of the growth material inhibited germination and plant establishment. This species has been observed in a small number of investigations to demonstrate tolerance to PTE and exhibit phytoremediation qualities (Zheljazkov and Nielsen 1996; Mashhoor Roodi *et al.* 2012; Parisa *et al.* 2014; Angelova *et al.* 2015), however the studies were completed using contaminated agricultural soils, suggesting that the species is dependent on an otherwise functioning soil system to be of use in phytoremediation. Therefore, at the trial sites this species would not be useful in future trials related to phytostabilisation. It was found that *C.pastoris* and *N.caerulescens* had the

highest aerial biomass, lowest root biomass and lowest surface cover in the group representing Le Bleymard. It was found that the root biomass for these species were not found to be significantly different between the control and spiked groups, suggesting that elevated PTE concentrations do not have a detrimental impact on root growth in these species. These species therefore appear to promote the growth of above ground biomass, an expected trait when considering their classification as *Brassicales*, rather than *Poaceae*, which encompass *A.capillaris*, *A.vinealis*, and *F.ovina*. This predisposition for the development of aerial biomass has likely made *C.pastoris* and *N.caerulescens* suitable candidates for phytoextraction (Aksoy *et al.* 1999; Gonneau *et al.* 2014), however the lower root biomass and surface cover would not make these species suitable for phytostabilisation, where a carpet layer of vegetation and a dense rooting system is desired (Hakeem 2015).

In contrast, *A.capillaris*, *A.vinealis*, and *F.ovina* were found to exhibit higher root biomasses and greater percentage surface cover than the *Brassicales*, with no observable differences between species or within treatment groups. This suggests that these three candidates may be useful as phytostabilisation options at the Le Bleymard mine tailings, based on initial findings that agree with Hakeem (2015).

In the synthesised Tyndrum tailing material *C.pastoris* was again found to exhibit greater shoot biomass than the *Poaceae* species, together with *C.hirsuta* which also exhibited higher shoot biomass. This difference may again be explained by their classification as *Brassicales* and the tendency to focus resources on aerial development to complete their life cycles as flowering plants. In contrast to the Le Bleymard group, the roots of *C.pastoris* were not significantly different to the *Poaceae* species, suggesting that the plant may grow better in slightly acidic environments. Considering the other species, root biomass was lowest in both treatments of *C.hirsuta*, suggesting that this plant has a low root biomass development regardless of PTE burdens.

Similarly to the Le Bleymard group, the majority of the *Poaceae* species exhibited high root biomass, with no significant differences between spiked and

control groups. This suggests that PTE presence does not affect root biomass production at the values tested, providing evidence to support the use of these species as potential phytostabilisers. Conversely, both *M.caerulea* and *A.vinealis* was found to have significantly greater root production and surface cover (*M.caerulea* only) in the control group, suggesting that these species may be detrimentally impacted by Pb and Zn. Therefore, *M.caerulea* may not be suitable for the revegetation of the Tyndrum tailings due to its apparent sensitivity to either Zn and/or Pb. This is in contrast to other work, where *M.caerulea* has been found to tolerate 4,600 and 20,600 mg kg⁻¹ of Pb and Zn in the root system respectively (Barrutia *et al.* 2011) and has been identified as a potential phytostabilisation species due to a dense rooting system and support for micro-organisms (Baba *et al.* 2016). However, Ryszka and Turnau, (2007) observed that seedling germination and survival was low within this species when grown directly on Pb/Zn tailings, rather than transplantation (Turnau *et al.* 2012), which may explain the reduced root biomass and surface cover witnessed in this study. Therefore, *M.caerulea* would not be suitable for this investigation as it is desirable for seeds to be grown directly on site to minimise remediation costs.

Surface cover was lowest in the pots containing *C.pastoris* and *C.hirsuta*, which when coupled with low root production in the latter species would not make either suitable candidates for phytostabilisation. A dense rooting system and continuous vegetative cover would be required to reduce tailing erosion and movement, which in the case of *C.pastoris*, has been previously identified to be low (Suchkova *et al.* 2014). Furthermore both species have been previously identified as displaying hyperaccumulator traits (De Haro *et al.* 2000; Grabner *et al.* 2011; Liu *et al.* 2014), which are undesirable traits for phytostabilisation goals due to increased translocation of PTE to aerial compartments of a plant and a focus on aerial biomass production over root systems.

Direct consideration between the root biomass of the control and spiked *A.vinealis* in the synthesised Tyndrum tailing material demonstrates that the spiked individuals display a significantly lower root biomass. This suggests that, similarly to *M.caerulea*, this species is negatively impacted by Zn and/or Pb

concentrations and may not be as resistant as compared to *A.capillaris* and *F.ovina* where no significant negative impacts were found. Data on the phytoremedial properties of *A.vinealis* are limited however, and describe mixed results in PTE resistance (Baker and Proctor 1990; Pichtel and Salt 1998; Pichtel and Bradway 2008) and so there is no conclusive evidence to support the use of this species as a phytostabilisation tool.

5.3.3 Conclusion: Phase 1

Based on the initial screening it appears that *A.capillaris* and *F.ovina* would be the most suitable species to take forward into a more detailed study at both sites. These species were established on the tailings at Tyndrum and Le Bleymard whist exhibiting high percentage cover and high root biomass production in the initial study. Therefore, the potential of *A.capillaris* and *F.ovina* as phytostabilisation tools should be investigated further for phytostabilisation suitability at these sites.

The *Brassicales* (*C.pastoris*, *C.hirsuta* and *N.caerulescens*) would not be suitable candidates for phytostabilisation of either site due to their lower root production and surface cover compared to the *Poaceae* groups. Large rooting biomass is essential for phytostabilisation purposes and for the intention of reducing PTE enriched tailing material transportation from the site (Hakeem, 2015). Moreover, despite the greater production of above ground biomass, these species exhibited a poor surface cover area, increasing exposed mine tailing material to aeolian and hydrological erosion in comparison to the grass species.

From this investigation *A.capillaris* and *F.ovina* were used in a secondary study (phase 2) to study the translocation of Pb and Zn within the plant tissues based on the discussions outlined in this section.

5.4 Phase 2: PTE Partitioning in *A.capillaris* and *F.ovina*

5.4.1 Shoot and Root Concentrations of Pb and Zn in *A.capillaris* and *F.ovina*

Concentrations of Pb found in the roots and shoots of *A.capillaris*, *F.ovina* and the mixed group are detailed in Figure 5-4. Concentrations of Pb in the spiked and control root tissues of *A.capillaris*, *F.ovina* and the mixed treatment were significantly higher than those found in the shoot ($p = < 0.001$). The control group for all species was found to have significantly lower Pb in the roots and shoots compared to the spiked treatment ($p = < 0.001$). No significant differences were seen in Pb concentration between species in either the control or spiked treatments ($p = > 0.05$).

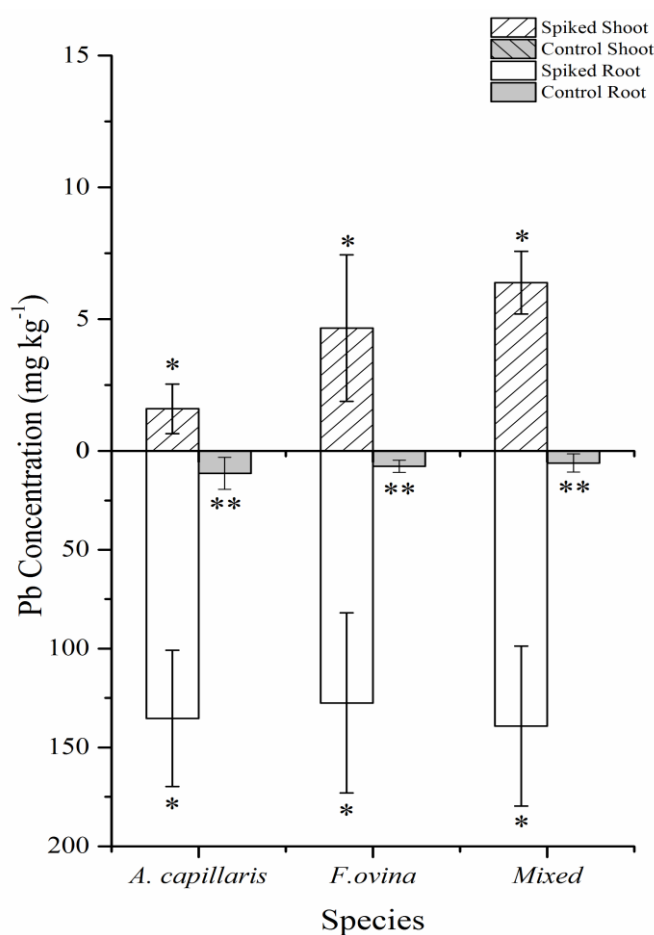


Figure 5-4. Root and shoot concentrations of Pb in spiked and control groups of *A.capillaris*, and *F.ovina* and the mixed group.

Similarly to Pb, Zn concentrations were significantly higher in the plant tissues grown in the spiked soil compared to the control ($p = <0.001$) (Figure 5-5). In the spiked treatment, root concentrations were significantly higher than the shoots for all groups ($p = 0.016$). No significant differences in the shoot or roots concentrations were observed between species or the mixed group in the spiked treatment ($p = > 0.05$).

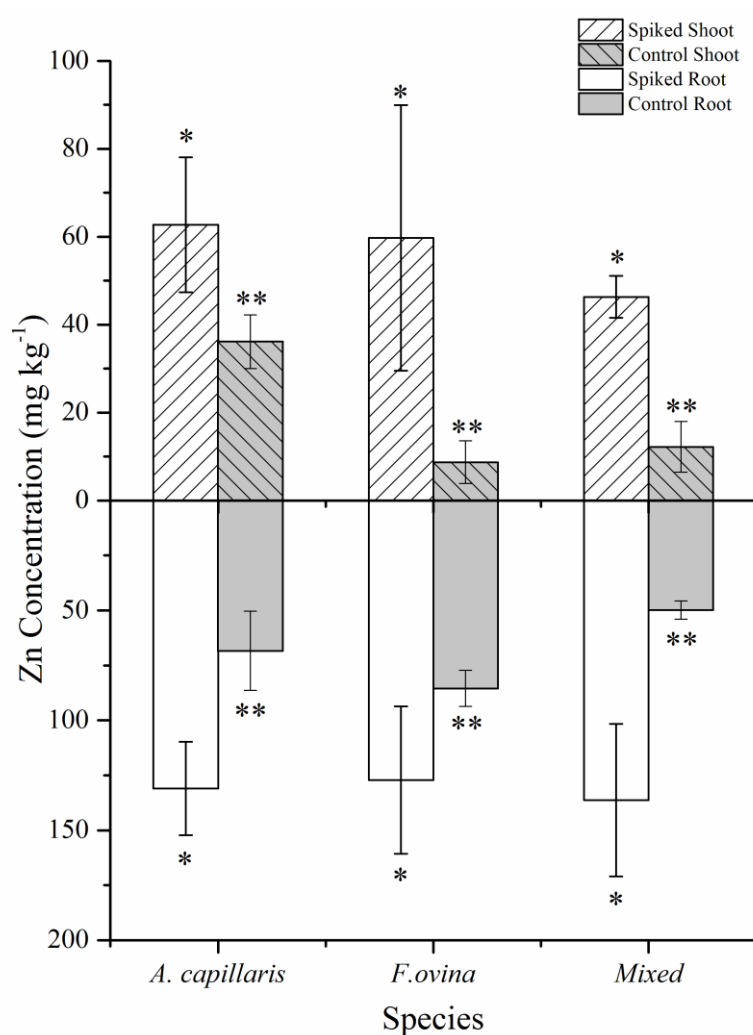


Figure 5-5. Root and shoot concentrations of Zn in spiked and control groups of *A. capillaris*, *F. ovina* and the mixed group.

The relative proportions (%) of Pb and Zn in the roots and shoots of both the control and the spiked groups are detailed in Table 5-2. For *A. capillaris* and the mixed group, partitioning of Pb and Zn in the roots and shoots were not significantly different between the spiked and control groups ($p = > 0.05$),

whereas in *F.ovina*, Zn translocation to the shoots was significantly increased in the spiked treatment compared to the control ($p = 0.009$).

Table 5-2. Relative proportions (%) of Pb and Zn in the roots and shoots of plant species in the spiked and control treatments. $n = 7$.

Spiked Group					Control Group			
Species	<u>Pb</u>		<u>Zn</u>		<u>Pb</u>		<u>Zn</u>	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
<i>A.capillaris</i>	1.2	98.8	32.3	67.7	<1%	> 99%	35.5	64.5
<i>F.ovina</i>	3.7	96.3	32.1	67.9	<1%	> 99%	8.1	91.9
<i>Mixed</i>	7.2	92.8	26.1	73.9	<1%	> 99%	18.2	81.8

5.4.2 Root and Shoot Biomass of Spiked and Control Groups

The biomass of shoots in all species and treatment groups were not significantly different ($p = 0.23$) whereas both spiked and control groups of *F.ovina* was found to have significantly lower root biomass than the control groups of *A.capillaris* and the mixed group ($p = 0.02$) Figure 5-6. No significant differences were observed between the root biomasses of the spiked and control groups within species ($p = 0.94$).

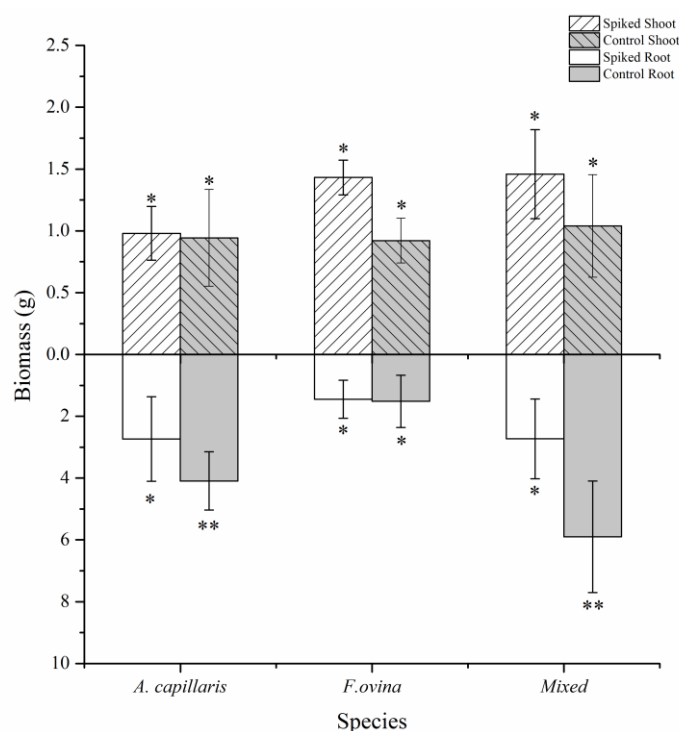


Figure 5-6. Root and shoot biomass of spiked and control groups.

5.4.3 Bioaccumulation Coefficient, Extraction Efficiency and Translocation Factors

The determination of bioaccumulation coefficients (BC), extraction efficiencies (EE) and translocation factors (TF) for each spiked group is given in Table 5-3. No significant differences were found in terms of BC for either Pb or Zn between groups ($p = 0.7$), however, translocation factors for Pb were significantly lower in *A. capillaris* compared to *F. ovina* and the mixed treatment ($p = 0.004$). No significant differences were observed in translocation factors for Zn between groups ($p = 0.47$). Finally, no significant differences in EF was observed between Pb or Zn in any species ($p > 0.05$).

Table 5-3. Mean Bioaccumulation coefficients (BC), translocation (TF) factors and extraction efficiencies (EE) for Pb and Zn for the spiked groups. $n = 7$.

Species	Pb BC	Zn BC	Pb TF	Zn TF	Pb EE	Zn EE
<i>A. capillaris</i>	0.015	0.017	0.012	0.43	0.16	0.18
<i>F. ovina</i>	0.015	0.018	0.038	0.55	0.15	0.19
Mixed	0.017	0.017	0.085	0.35	0.17	0.18

5.4.4 Total Nitrogen Content in Shoots

The total nitrogen concentration in the control shoots of *A.capillaris* were 0.76 % higher than those in the spiked treatment ($p = 0.024$). No significant differences were seen observed between other species or treatments between spiked and control groups.

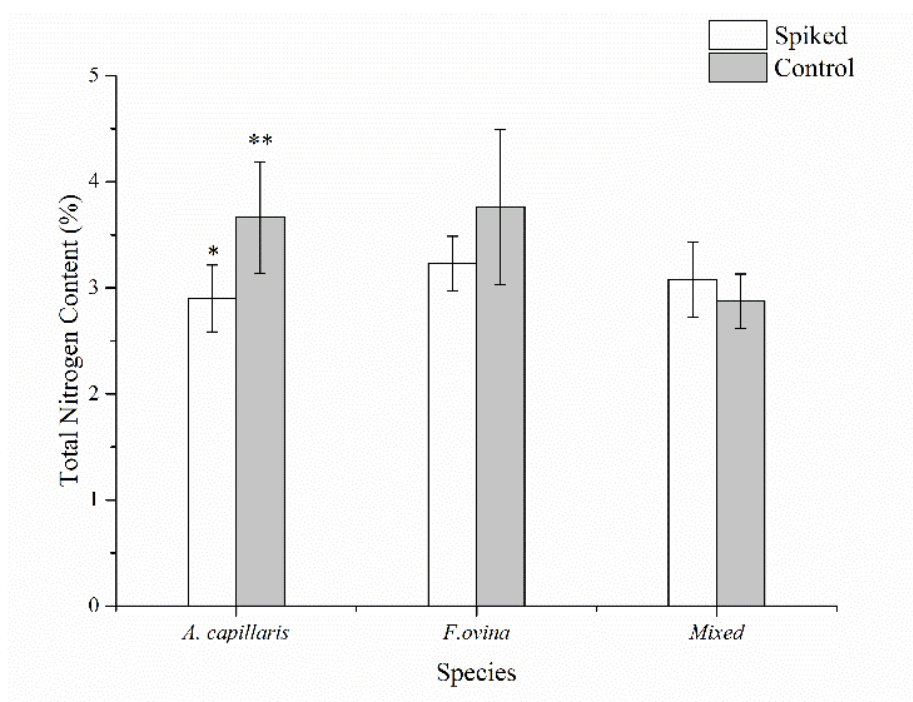


Figure 5-7. Total N content of spiked and control shoots in the phase 2 study.

5.4.5 Discussion Phase 2

The results from this chapter demonstrate that *A.capillaris*, *F.ovina* and a combination of the two species all have useful characteristics in terms of phytostabilisation.

Lead in all three groups were mainly sequestered in the roots, with a mean of 1.2, 3.7 and 7.2 % of the plant loading of Pb translocated to the aerial compartments of the plants for *A.capillaris*, *F.ovina* or the mixed group respectively. Furthermore, extraction efficiencies for all plant treatments and both Pb and Zn were less than 0.2%. Both of these metrics imply a low affinity for bioaccumulation of PTE from the soil system. Criteria for an ideal species for phytostabilisation is debated, but often include high biomass, dense rooting

systems and low uptake or translocation of PTEs to the aerial compartments of the plants (Singh 2012; Ali *et al.* 2013; Ellili *et al.* 2017; Wang *et al.* 2017). The measure of ‘high biomass’ is often relative to other plant species in question, however all the groups tested in this investigation exhibit a dense rooting system and low translocation of Pb.

Furthermore, benchmarks for hyperaccumulating plants, or those which are to be used for PTE extraction purposes have been identified as those which can accumulate greater than 1,000 mg kg⁻¹ dry mass of Pb in the aerial compartments of the plant (Baker and Brooks 1989; Lasat 2002; van der Ent *et al.* 2013), and demonstrate a TF of greater than 1 (Tangahu *et al.* 2011), neither of which have been identified in either *A.capillaris*, *F.ovina* or the mixed group. Bioaccumulation coefficients for Pb in all species were less than 0.02, demonstrating a significantly lower value than other species studied for this element (Cheraghi *et al.* 2011; Rezvani and Zaefarian 2011; Sooksawat *et al.* 2013).

Considering *A.capillaris*, similar findings for low translocation of Pb have been reported in other studies (Table 5-4). In general, *A.capillaris* has been observed to exhibit low TF across several sites and environmental conditions for Pb, with the authors considering *A.capillaris* to be an excluder of Pb and therefore useful for phytostabilisation purposes. High TF factors found in Table 5-4 were attributed to differing qualities on the physio-chemical nature of the tested soil, the influence of bacteria and tolerant ecotypes of *A.capillaris*. Furthermore, Rodríguez-Seijo *et al.*, (2016) indicated bioaccumulation coefficients and translocation factors for Pb in *A.capillaris* were generally <1, indicating the species’ potential for phytostabilisation. Translocation and uptake of Zn was generally higher than Pb in this investigation and the studies detailed in Table 5-4, most likely due to the role of this element as an essential micronutrient and its function in several essential proteins in the shoot (Haydon 2014). Indeed, increasing Zn translocation is often directly linked to increased soil Zn and is common across several plant families (Broadley *et al.* 2007).

Zn concentrations in the shoots of *A.capillaris* in this study would not be expected to impact the health of grazing herbivores, suggesting that the species may be able to be utilised at the Tyndrum and Le Bleymard mines without significant impacts on ecological systems in the short term (Behmer *et al.* 2005; Coleman *et al.* 2005; Kazemi-Dinan *et al.* 2014).

Table 5-4. Reported translocation factors (TF) for Pb and Zn in *A.capillaris*.

Study	Pb TF	Zn TF
Langella <i>et al.</i> , (2014)	0.19	0.88
Nicoară <i>et al.</i> , (2014)	0.25	0.3
Perkins <i>et al.</i> , (2016)	0.04 – 2.12	N/A
Bech <i>et al.</i> , (2012)	0.2 – 1.0	0.32 – 0.35
Malcová <i>et al.</i> , (2003)	0.008 – 0.025	N/A
Doubková and Sudová, (2016)	2.27	0.52

Despite no significant differences observed in either the root or shoot biomass of *A.capillaris* in this study TN in the shoot was significantly lower in the spiked treatments as compared to the control. This may suggest that *A.capillaris* was under some stress from increased PTE burdens which was limiting N production in the shoot, and therefore plant development (Boussama *et al.* 1999; Athar and Ahmad 2002).

Festuca ovina and the mixed group in this study demonstrated higher translocation factors of Pb than *A.capillaris*, suggesting that this species may have a lower desirable phytostabilisation potential than *A.capillaris*. In the wider literature, *F. ovina* has been demonstrated to be tolerant to the environmental conditions of Pb/Zn wastes (Shimwell and Laurie 1972; Brown 1995; Yang *et al.* 2005b; Stefanowicz *et al.* 2010a; Bini *et al.* 2017), and has been observed to colonise approximately 40% of Pb/Zn tailings in a study by Marsili *et al.*, (2009). Moreover, a small number of studies have found that *F. ovina* can exclude Pb and Zn from plant tissues (Brown and Brinkmann 1992a; Álvarez *et al.* 2003; Charlier *et al.* 2005), with bioconcentration factors ranging from 0.01 – 0.15 for

Pb and 0.39 – 3.2 for Zn (Brown and Brinkmann 1992a; Charlier *et al.* 2005). However, there is evidence suggesting that *F.ovina* is often detrimentally impacted by elevated concentrations of CaCl₂ extractable Pb, Zn and Cd (1,000, 4,300 and 0.8 mg kg⁻¹ respectively), inhibiting root development (Garland and Wilkins 1981; Lago-Vila *et al.* 2017a) and suppressing the production of shoot biomass due to high translocation of PTE to the aerial compartments of the plant (Barry and Clark 1978; Kruger *et al.* 1997; Yanqun *et al.* 2005; Hernández-Allica *et al.* 2008). These concentrations however are typically higher than the concentrations estimated to be encountered at both Tyndrum and Le Bleymard. It is possible that the divergent findings in the literature regarding tolerance and translocation factors may be partly attributable to the use of PTE tolerant ecotypes and commercial seedlings in these studies (Urquhart 1971; Brown and Brinkmann 1992a), as well as soil-specific characteristics such as pH and organic matter content (Lago-Vila *et al.* 2017a).

In this study, although *F.ovina* demonstrated significantly higher translocation factors than *A.capillaris* for Pb, it could not be said that the species exhibited properties of hyperaccumulation (van der Ent *et al.* 2013) or displayed a decrease in root biomass development when exposed to high concentrations of soluble Pb and Zn. Furthermore, *F.ovina* did not accumulate significantly different concentrations of either Pb or Zn than *A.capillaris* or the mixed species group, suggesting that this species performs equally to the other species tested, despite the use of commercial seeds rather than specialised ecotypes (Urquhart 1971; Brown and Brinkmann 1992a). However, root biomass was observed to be significantly lower in both spiked and control groups of *F.ovina* compared to the control groups of *A.capillaris* and the mixed seed treatment, suggesting that the species does not generate a larger root system when unstressed, but does not respond negatively to heightened Pb and Zn burdens in the soil environment either. Although a greater translocation factor was observed for *F.ovina* the concentrations of Pb and Zn in the shoots would not be considered an ecological issue, based on the same arguments in the previous sections considering *A.capillaris* translocation (Behmer *et al.* 2005; Coleman *et al.* 2005; Kazemi-Dinan *et al.* 2014).

The mixed species treatment comprising of a 1:1 ratio of *A.capillaris* and *F.ovina* did not display any significant differences from the measured variables from each individual treatment, with the exception of a diminished rooting system in the spiked treatment compared to the control. Despite this, root biomass in the spiked group was not significantly different to either spiked groups of *A.capillaris* and *F.ovina*, and total shoot N was not observed to be significantly reduced, suggesting that a mixed planting system can work as effectively as a monoculture system. In general greater biodiversity in plant species can support a more complex and diverse soil environment, and so the use of a mixed species in terms of phytoextraction may be more desirable (Grayston *et al.* 1998; Kowalchuk *et al.* 2002; Berg and Smalla 2009; Ladygina and Hedlund 2010).

5.4.6 Conclusion of Phase 2

Agrostis.capillaris, *F.ovina*, and a 1:1 mixture of both species has demonstrated low bioaccumulation and translocation of Pb and Zn into the root system and aerial compartments of the plants, leading to a low assessed risk of Pb and Zn transportation in food chains via herbivorous grazing. Furthermore, despite evidence for diminished rooting capacity and some evidence of N stress in *A.capillaris*, all tested groups performed equally in terms of biomass production in both roots and shoots, supporting the proposition that *A.capillaris*, *F.ovina* and a 1:1 mixture could be useful phytostabilisation candidates. It was suggested that the mixed seed composition was to be used in the field trials in Chapter 7 to promote the opportunity of a more microbially diverse soil system at the tailing heaps at Le Bleymard and Tyndrum (Berg and Smalla 2009).

5.5 Chapter Conclusions

This chapter has investigated the phytostabilisation potential of eight species of plants found at the mine tailings at Tyndrum and Le Bleymard. The initial screening survey was successful in highlighting two species (*A.capillaris* and *F.ovina*) found to naturally colonise both mines that exhibited a tolerance to elevated concentrations of PTE, low nutrient content and poor structural support.

In phase 2, these species were exposed to elevated loadings of soluble Pb and Zn to assess their tolerance, uptake and translocation of PTE to the aerial compartment of the plant. From phase 2 it was found that both *A.capillaris* and *F.ovina* demonstrated a low uptake of Pb and Zn, combined with a low translocation of Pb to the shoot biomass, indicating that these species would be useful candidates to bring forward to the field trials described in Chapter 7.

Finally, this chapter has contributed to the wider scientific literature by screening plant species which may or may not be useful in the re-vegetation of calcareous and slightly acidic tailing heaps. Furthermore, the chapter has expanded the evidence of Pb and Zn tolerance in *A.capillaris* and *F.ovina*, supporting their potential use as phytostabilisation tools for PTE contaminated sites.

Chapter 6. Organic Amendment Incorporation into the Tyndrum and Le Bleymard Tailings

6.1 Introduction

This chapter will focus on the incorporation of four organic amendments into the tailing material sourced from Tyndrum and Le Bleymard. Each organic amendment was mixed into the respective tailing material at two application rates and assessed for changes in biological, physical and chemical parameters over a four-week period.

This chapter will be split into the following three sections: i) The impacts to the nutrient content and availability, microbial functioning and general changes in tailing characteristics. ii) Evaluation of tailing properties in terms of PTE mobility and potential transport mechanisms and iii) A comparison of the changes in PTE availability in the solid phase of the amended and un-amended tailings.

Each section will present results from columns containing tailing material from Tyndrum and Le Bleymard concurrently, allowing comparisons and discussions to be drawn from the results. A final discussion will assemble the collected results to form holistic conclusions on the findings for both Pb/Zn mines, informing the planning process of the field studies and highlighting the contributions of the work to the scientific field.

6.2 Rationale/Overview

The findings presented in this chapter were essential for informing the field trials detailed in Chapter 7 by identifying which organic amendments may be best suited for each site and avoiding those which may exacerbate PTE mobility or bioavailability. As both mine tailings are located in National Parks it was essential to have prior knowledge on the possible implications of adding organic amendments into the tailing material to avoid unexpected reactions and to maximise the effectiveness of the field trials.

This investigation presents a novel and holistic investigation into the impacts of incorporating organic amendments into mine tailings by considering gaseous

fluxes, soil pore water and the solid phase simultaneously. This chapter provides new information on risks and benefits of bioremediation approaches and highlights the substantial variation in impacts organic amendments can have when applied to dissimilar mine tailings.

6.3 Methods and Statistical Approaches

6.3.1 Method Summary

A full overview of the experiment can be found in Chapter 3, section 3.4.

For each replicate, approximately 0.5 kg of mine tailing material sourced from either Tyndrum or Le Bleymard were separately mixed with low (8% total mass) and high (16% total mass) applications of cattle manure (CM), sheep manure (SM), green waste compost (GWC) and spent coffee grounds (SCG) respectively. The amended material was packed into 300 mm x 45 mm diameter leaching columns with sealable lids to collect gas and leachate over a four-week period. These were maintained at a stable temperature of 18°C and maintaining a 60% water holding capacity. In total, 36 columns were constructed for each mine site, consisting of 4 replicates for each treatment group. The leachate was analysed for a range of parameters (Total carbon (TC), Eh, P, pH and NO_2^- -N+ NO_3^- -N/ NH_4^+ -N, PTE, and other elements) and CO_2 fluxes were also determined (Chapter 3).

At the end of the four-week period the columns were dismantled, and the solid phase analysed for pH, NO_2^- -N+ NO_3^- -N/ NH_4^+ -N, MBC, TC, TN, water soluble P, LMWOA-extractable PTE, and in the case of the Tyndrum mine tailing material, BCR-extractable Pb, Cd and Zn. The BCR procedure was not suitable for assessing the Le Bleymard samples due to pH buffering issues (Appendix 1), and so only LMWOA extractable PTE concentrations were assessed.

The chemical parameters of the organic amendments are given in (Table 6-1). Cattle and SM were characterised by high pH, NH_4^+ - N, water soluble P and total N in comparison to SCG and GWC. All amendments contained elevated concentrations ($>15,000 \text{ mg kg}^{-1}$) of CWEC/HWEC in the exception of GWC.

Concentrations of PTEs (As, Zn, Pb, Cd) were generally low and were not expected to significantly enrich the baseline tailing concentrations.

Table 6-1. Properties of organic amendments used in chapters 6 and 7. CM = Cattle Manure, SM = Sheep Manure, GWC = Green waste compost, SCG = Spent Coffee Grounds. n = 6

Parameter	CM	SM	COM	SCG
pH (range)	7.9 - 8.1	9.1 - 9.4	7.45 - 7.51	5.25 - 5.35
LOI (%)	85.6 (\pm 0.6)	83.2 (\pm 0.2)	12.1 (\pm 0.3)	98.7 (\pm 0.5)
NO ₃ ⁻ - N (mg kg ⁻¹)	12.9 (\pm 0.3)	4.9 (\pm 0.3)	163 (\pm 20)	6.5 (\pm 0.4)
NH ₄ ⁺ - N (mg kg ⁻¹)	98.2 (\pm 3.2)	237 (\pm 4)	35.6 (\pm 1.1)	62.5 (\pm 1.4)
Total N (%)	2.72 (\pm 0.05)	3.35 (\pm 0.05)	0.46 (\pm 0.02)	2.07 (\pm 0.06)
CWC (mg kg ⁻¹)	18,900 (\pm 996)	21,900 (\pm 496)	332 (\pm 17)	41,600 (\pm 470)
HWC (mg kg ⁻¹)	18,300 (\pm 402)	24,900 (\pm 966)	3,800 (\pm 329)	28,700 (\pm 3,800)
Total C (%)	38.0 (\pm 0.83)	37.0 (\pm 1.1)	8.9 (\pm 0.4)	46.7 (\pm 1.1)
C:N Ratio	13:1	11:1	19:1	22:5
Total As (mg kg ⁻¹)	0.62 (\pm 0.47)	0.35 (\pm 0.13)	0.76 (\pm 0.27)	0.009 (\pm 0.013)
Total Ca (mg kg ⁻¹)	11,900 (\pm 176)	16,700 (\pm 23.2)	9,900 (\pm 339)	1,146 (\pm 35.1)
Total Cd (mg kg ⁻¹)	<0.001	<0.001	<0.001	<0.001
Total Cu (mg kg ⁻¹)	20.7 (\pm 0.6)	35.3 (\pm 0.94)	48.9 (\pm 1.7)	23.1 (\pm 0.2)
Total Fe (mg kg ⁻¹)	1,440 (\pm 130)	1,580 (\pm 0.94)	20,400 (\pm 640)	162 (\pm 90)
Total Mg (mg kg ⁻¹)	5,350 (\pm 126)	8,640 (\pm 0.94)	3,340 (\pm 98)	1,380 (\pm 21)
Total Mn (mg kg ⁻¹)	1,441 (\pm 28)	1,246 (\pm 27)	464 (\pm 17)	39.6 (\pm 0.2)
Total P (mg kg ⁻¹)	4,200 (\pm 99)	5,640 (\pm 6.3)	1,390 (\pm 50)	1,030 (\pm 16)
Total Pb (mg kg ⁻¹)	3.6 (\pm 0.04)	17.6 (\pm 1.3)	68.9 (\pm 1.1)	< 0.006
Total S (mg kg ⁻¹)	1,795 (\pm 25)	1,962 (\pm 100)	768 (\pm 26)	535 (\pm 20)
Total Zn (mg kg ⁻¹)	107 (\pm 1.6)	267 (\pm 16)	238 (\pm 3.9)	12.9 (\pm 0.6)
Water-soluble Fe (mg kg ⁻¹)	156 (\pm 12.3)	223 (\pm 23.9)	16.4 (\pm 3.7)	8.8 (\pm 2.5)
Water-soluble Mn (mg kg ⁻¹)	5.6 (\pm 0.2)	4.3 (\pm 1.8)	8.27 (\pm 0.13)	18.3 (\pm 4.4)
Water-soluble P (mg kg ⁻¹)	332 (\pm 41.8)	673 (\pm 51.9)	99.4 (\pm 13.3)	66.5 (\pm 10.8)

6.3.2 Statistical Approaches

General Data Management, Statistical Packages and Graphing Software

Collected data was tested for normality by visually assessing histograms and applying the Anderson-Darling test for data to be assessed by parametric models. For the detection of outliers Grubbs' test was used. Data that required testing via parametric means were log-transformed where necessary to meet the requirements of normality. All tests were performed at the significance level of $\alpha = 0.05$

The majority of statistical testing was performed in Minitab v.18, with graphical outputs produced using Origin 2016. Principal Component Analysis (PCA) was completed using RStudio and R Version 3.4.2. The script for PCA in R is given in Appendix 3.

Full outputs of the statistical tests can be found in Appendix 4, 5 and 6.

Gas and Leachate Data

Data collected over the four-week period were assessed using a repeated measures ANOVA and at each sampling week a one-way ANOVA with Tukey grouping was completed to ascertain differences in treatment groups at each sampling interval. When comparing treatment groups across both Tyndrum and Le Bleyard a Two-way ANOVA model was used. Descriptive statistics including, but not limited to, the mean, standard deviation and range were also determined. Principal Component Analysis was undertaken on the measured parameters in the leachate using RStudio.

Solid Phase

One (with Tukey grouping) and Two-way ANOVA were used to determine significant differences between treatments and tailing groups in the determination of pH, NO_2^- -N+ NO_3^- -N/ NH_4^+ -N, MBC, TC, TN, water soluble P, and LMWOA-extractable PTE. To assess significant differences in the BCR extractions an Analysis of Means (ANOM) approach was used to highlight potential interactions between treatment applications and the operationally defined fractions.

6.4 Tailing Nutrients and Microbial Activity

6.4.1 Gas fluxes

6.4.1.1 CO₂ fluxes in the Le Bleymard Group

Weekly CO₂ fluxes for the treated and un-amended columns are shown in Figure 6-1. All organic materials and their respective high and low application rates increased the weekly CO₂ flux as compared to the un-amended columns across the four-week period ($p = <0.001$).

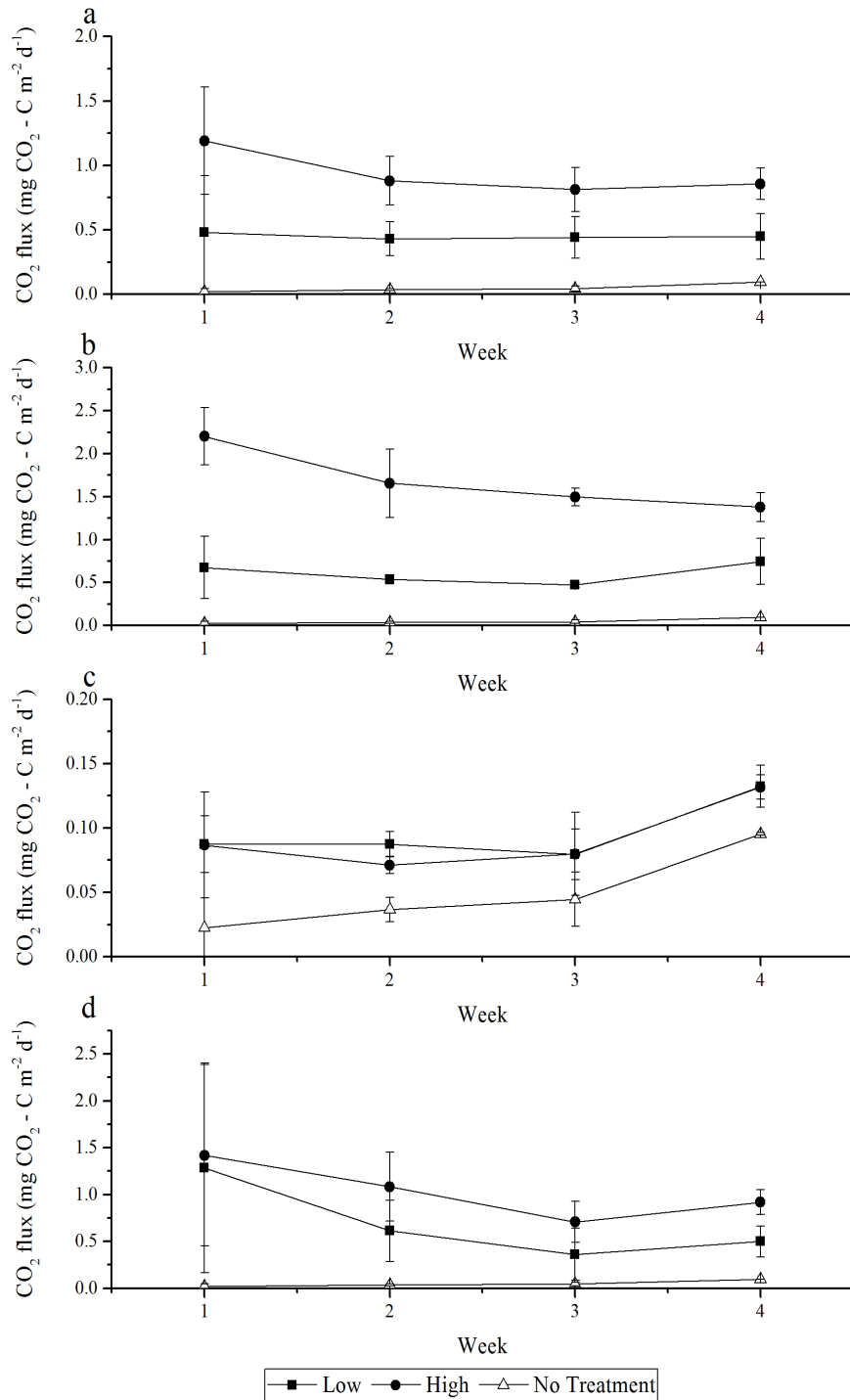


Figure 6-1. Mean Weekly CO₂ fluxes from high, low and non-treated columns for CM (a), SM (b), GWC (c) and SCG (d) of the Le Bleymard tailings. n = 4

The high application of CM elicited 0.5 (± 0.1) mg CO₂ – C more than the low application rate and 1.2 (± 0.8) mg CO₂ – C more than the un-amended tailings

per m^2/d^{-1} ($p = <0.001$) (Figure 6-1a). No significant differences in the fluxes within applications between weeks 1-4 were observed ($p = 0.59$).

High applications of SM produced a significantly greater CO_2 flux than the lower treatment in week 1 compared to weeks 3 and 4 ($p = 0.021$), (Figure 6-1b). In the low SM treatment, no significant differences in CO_2 flux were observed over the 4 weeks ($p = 0.781$). Over the experimental period high application rates of SM increased CO_2 flux by $1.6 (\pm 0.4)$ and $0.98 (\pm 0.4)$ $\text{mg CO}_2 - \text{C m}^2/\text{d}^{-1}$ as compared to the un-amended tailings and low SM treatment respectively. Low application rates increased CO_2 fluxes by $0.66 (\pm 0.04)$ $\text{mg CO}_2 - \text{C}$ as compared to the un-amended tailings.

For the GWC treatment, no significant differences were observed between either the high or low application rates in weeks 1-3 ($p = 0.338$) (Figure 6-1c). However, both treatments were had a significantly higher CO_2 flux in week 4 compared to weeks 1 to 3 ($p = <0.001$). Over the four-week period low and high application of GWC increased CO_2 flux by $0.04 (\pm 0.02)$ $\text{mg CO}_2 - \text{C m}^2/\text{d}^{-1}$ as compared to the un-amended tailings.

Spent coffee grounds (Figure 6-1d) did not produce significantly different CO_2 emissions between weeks 1-4 and between the high and low application rates ($p = 0.16$), with the exception of week 4 where the high application rate of SCG was significantly higher than the lower treatment ($p = 0.007$).

In week one both GWC treatments had significantly lower CO_2 fluxes than all other treatment groups ($p = <0.001$), with no other significant differences observed between organic amendment types or application rates ($p = > 0.05$). In the second week CO_2 fluxes in the high treatments of SM, CM and SCG were found to be significantly elevated as compared to the un-amended tailings, both GWC treatments, and the low application of CM ($p = <0.05$). The high application rate of SCG increased CO_2 flux $0.98 (\pm 0.31)$ $\text{mg CO}_2 - \text{C m}^2/\text{d}^{-1}$ as compared to the un-amended tailings, with the low application demonstrating a $0.6 (\pm 0.4)$ increase.

In the third week CO₂ emissions in the high SM treatment was significantly higher than all low treatments of organic amendments and the high and low GWC treatments ($p = <0.05$). Both GWC and the un-amended columns had significantly lower CO₂ emissions than all other treatments ($p = <0.05$). In the fourth and final week all application rates of all organic amendments had significantly higher CO₂ fluxes than the un-amended tailings, with SCG, CM and SM exhibiting significantly higher CO₂ emissions than both high and low GWC treatments ($p = <0.05$). Furthermore, no significant differences between types organic amendments were observed at the low or high treatments respectively in week four ($p = >0.05$). However higher application rates were found to elicit a greater emission of CO₂ per m²/d⁻¹ than the lower rate ($p = <0.05$).

In general, SM had the highest weekly fluxes of CO₂, followed by CM, SCG and finally GWC treatments, producing emissions several orders of magnitude higher than the un-amended tailings. Furthermore, higher application rates of the respective organic amendments were generally observed to increase CO₂ emissions in comparison to the lower rate. Fluxes were highly variable, particularly in week 1 and generally decreased or remained constant over time, with the exception of the GWC treatments where a significant increase in respiration was observed.

6.4.1.2 CO₂ fluxes in the Tyndrum Group

Carbon dioxide fluxes in the amended Tyndrum tailings were highly variable depending on the organic amendment and application rate used (Figure 6-2). Cattle manure applications were found to produce significantly higher CO₂ fluxes in the high application rate as compared to the un-amended tailings, with a $0.3 (\pm 0.15)$ mg CO₂ – C m²/d⁻¹ increase on average each week ($p = <0.001$) (Figure 6-2a). Low application rates were found to be on average $0.13 (\pm 0.08)$ mg CO₂ – C m²/d⁻¹ higher than the un-amended tailings, with only a significant increase observed in week 2 ($p = 0.03$).

Sheep manure increased CO₂ fluxes in both the high and low application rates as compared to the un-amended tailings, with an average increase of $1.02 (\pm 0.46)$ and $0.34 (\pm 0.16)$ mg CO₂ – C respectively ($p = <0.001$) (Figure 6-2b).

Furthermore, higher applications were found to promote a greater flux of CO₂ with a mean increase of 0.64 (\pm 0.4), ($p = <0.001$).

Carbon dioxide fluxes in the GWC amended mine tailing material were not significantly different in either high or low treatment group or the un-amended tailings ($p = 0.942$). High variation was observed in the weekly emission of CO₂ from this treatment group between replicates.

Carbon dioxide fluxes from SCG treated tailings (high and low applications) were significantly higher than from the control tailings throughout the experiment (Figure 6-2d, $p = <0.001$). Calculated fluxes peaked at weeks two and three, with a higher application of SCG yielding greater CO₂ flux as compared with both the un-amended tailings and the lower SCG application rate. Respiration rates at the beginning and conclusion of the experimental period were not significantly different in either treatment ($p = >0.05$).

Throughout the experimental period fluxes resulting from high applications of SM and SCG were found to be significantly higher than both GWC treatments and the un-amended tailings ($p = <0.05$). From week two onwards both application rates of CM and SM, and both SCG treatments elicited a greater CO₂ flux as compared with the un-amended tailings ($p = <0.05$).

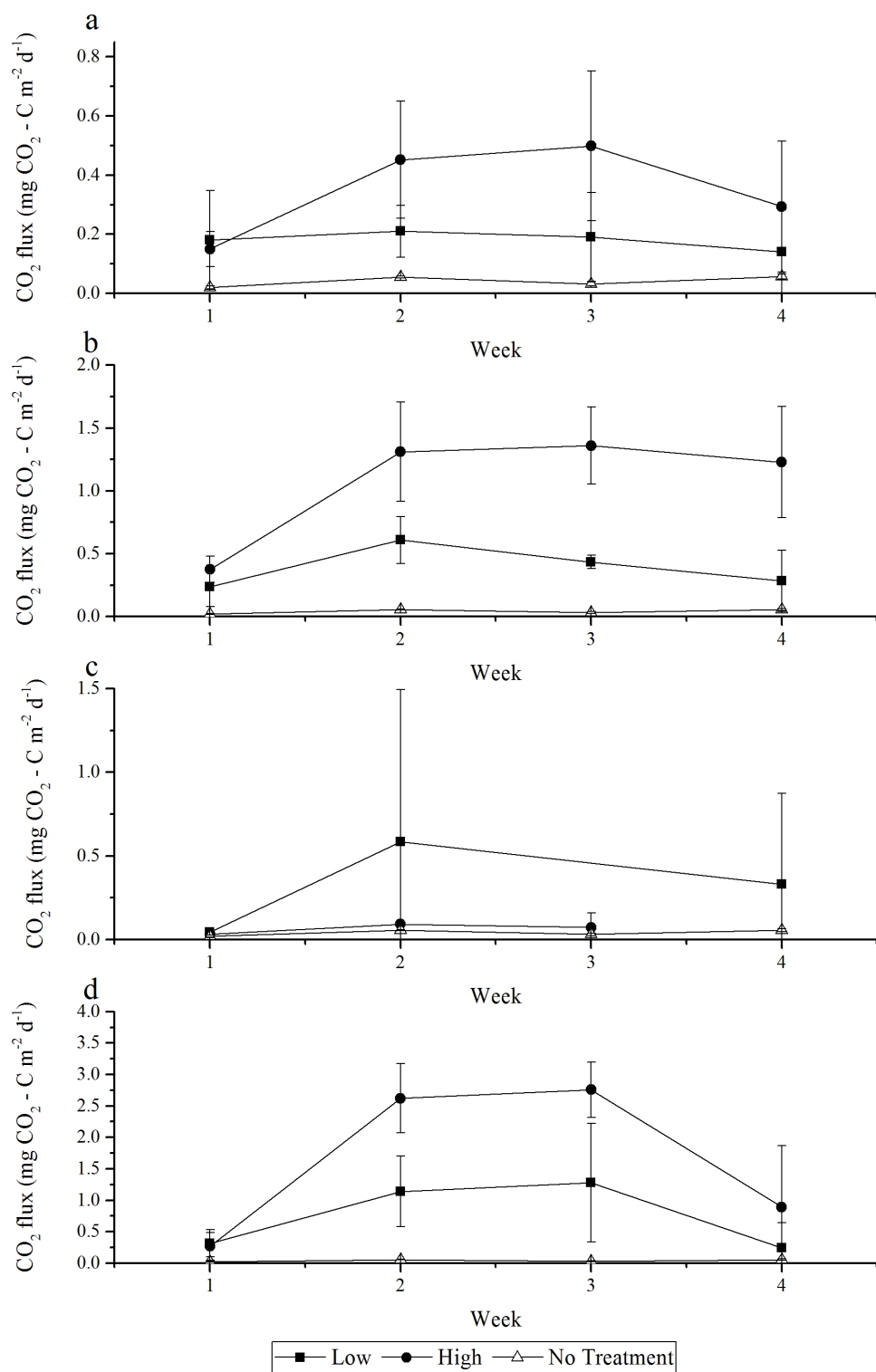


Figure 6-2. Mean Weekly CO₂ fluxes from high, low and non-treated columns for CM (a), SM (b), GWC (c) and SCG (d) of the Tyndrum tailings. n =4

6.4.1.3 Comparison of CO₂ fluxes between Le Bleymard and Tyndrum

Mean CO₂ fluxes over the four-week period for each site are compared (Figure 6-3). Significant differences were found between high and low applications of CM, with greater CO₂ flux observed in the Le Bleymard tailings as compared to Tyndrum ($p = <0.05$). No other treatments were found to be significantly different between sites ($p = >0.05$). For both sites, SCG, and SM promoted greater CO₂ fluxes as compared to the other amendments, with CM and the low application rates of SM and SCG observed to be statistically similar ($p = <0.05$). All organic amendments with the exception of both application rates of GWC applied to the Tyndrum tailings and the low application of GWC in the Le Bleymard tailing material increased CO₂ fluxes as compared to the un-amended tailings ($p = <0.05$).

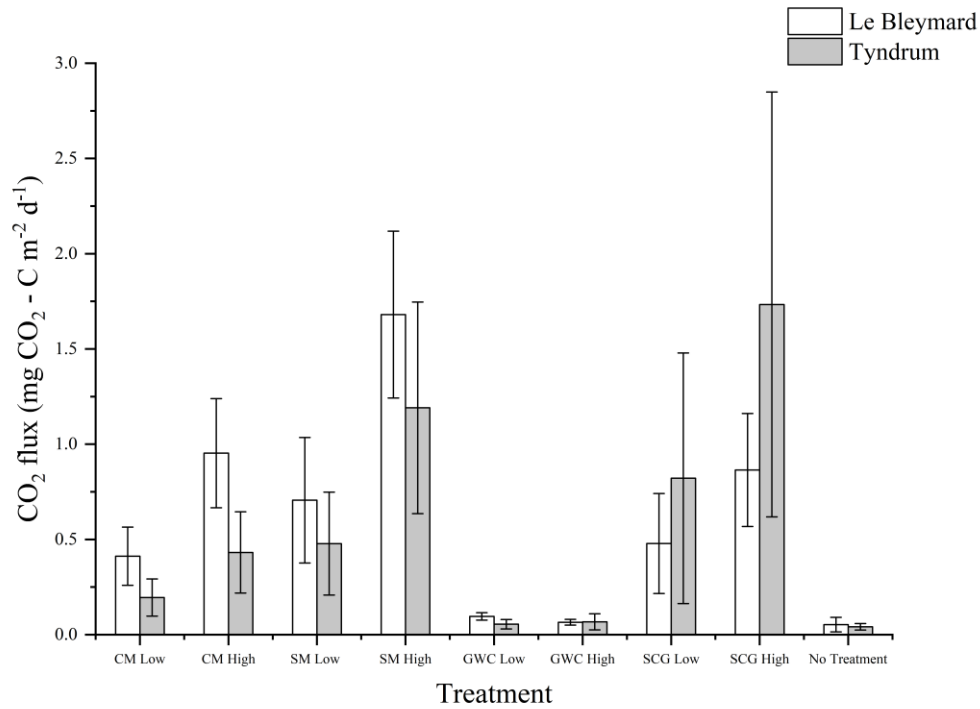


Figure 6-3. Mean CO₂ flux for each treatment over the four-week period in the tailings sourced from Tyndrum and Le Bleymard. CM = Cattle Manure, SM = Sheep Manure, GWC = Green waste compost, SCG = Spent coffee grounds. n = 4

6.4.2 Nitrogen and Phosphorous Behaviour

6.4.2.1 Weekly N leaching in the Le Bleymard Group

Concentrations of soluble, oxidised N ($\text{NO}_2^- + \text{NO}_3^-$) and ammonium (NH_4^+) collected from the leached columns with the Le Bleymard and Tyndrum tailings columns are illustrated in Figure 6-4 and Figure 6-5. In general, concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N had inverse trends, with $\text{NO}_2^- + \text{NO}_3^-$ -N generally increasing and NH_4^+ -N decreasing throughout the experimental period. For all treatments initial leaching of both forms of N were significantly higher than from the un-amended tailings ($p < 0.05$).

For CM and SM treatments there was a strong inverse relationship between $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N, with significant increases of $\text{NO}_2^- + \text{NO}_3^-$ -N between each collection period and a concurrent decrease in NH_4^+ -N ($p < 0.05$) (Figure 6-4a-b and Figure 6-5a-b). The application of SM led to significantly higher leaching of both forms of N in comparison with application of CM. The

exception was $\text{NH}_4^+\text{-N}$ at week 4 (Figure 6-5Figure 6-1a) ($p = <0.05$). In general, higher applications resulted in a greater leachate concentration of $\text{NO}_2^- + \text{NO}_3^- \text{-N}$ and $\text{NH}_4^+\text{-N}$ collected at each time period.

Green waste compost application gave high initial leachate concentrations of $\text{NO}_2^- + \text{NO}_3^- \text{-N}$ and $\text{NH}_4^+\text{-N}$ in week one, with a significant decrease in these forms of N in the second week ($p = <0.05$) (Figure 6-4c and Figure 6-5c). In weeks three and four leachate concentrations of $\text{NO}_2^- + \text{NO}_3^- \text{-N}$ and $\text{NH}_4^+\text{-N}$ were not significantly different from those for the un-amended tailings ($p = > 0.05$). For the SCG treatment, leaching of N declined with time, with concentrations of $\text{NO}_2^- + \text{NO}_3^- \text{-N}$ statistically indistinguishable from those for the un-amended tailings at week four (Figure 6-4d). Ammonium concentrations in leachates from SCG-treated tailing material remained significantly higher throughout the leaching period than the un-amended tailings ($p = <0.05$) (Figure 6-5d).

Comparing amendments, application of cattle and SM elicited significantly greater leachate concentrations of $\text{NO}_2^- + \text{NO}_3^- \text{-N}$ and $\text{NH}_4^+\text{-N}$ than SCG. However, GWC application gave an initially greater leaching of $\text{NO}_2^- + \text{NO}_3^- \text{-N}$ as compared with all other organic amendments. Unlike the manure treatments the application rates of GWC and SCG did not produce significantly different leachate concentrations of either forms of N throughout the experimental period ($p = >0.05$).

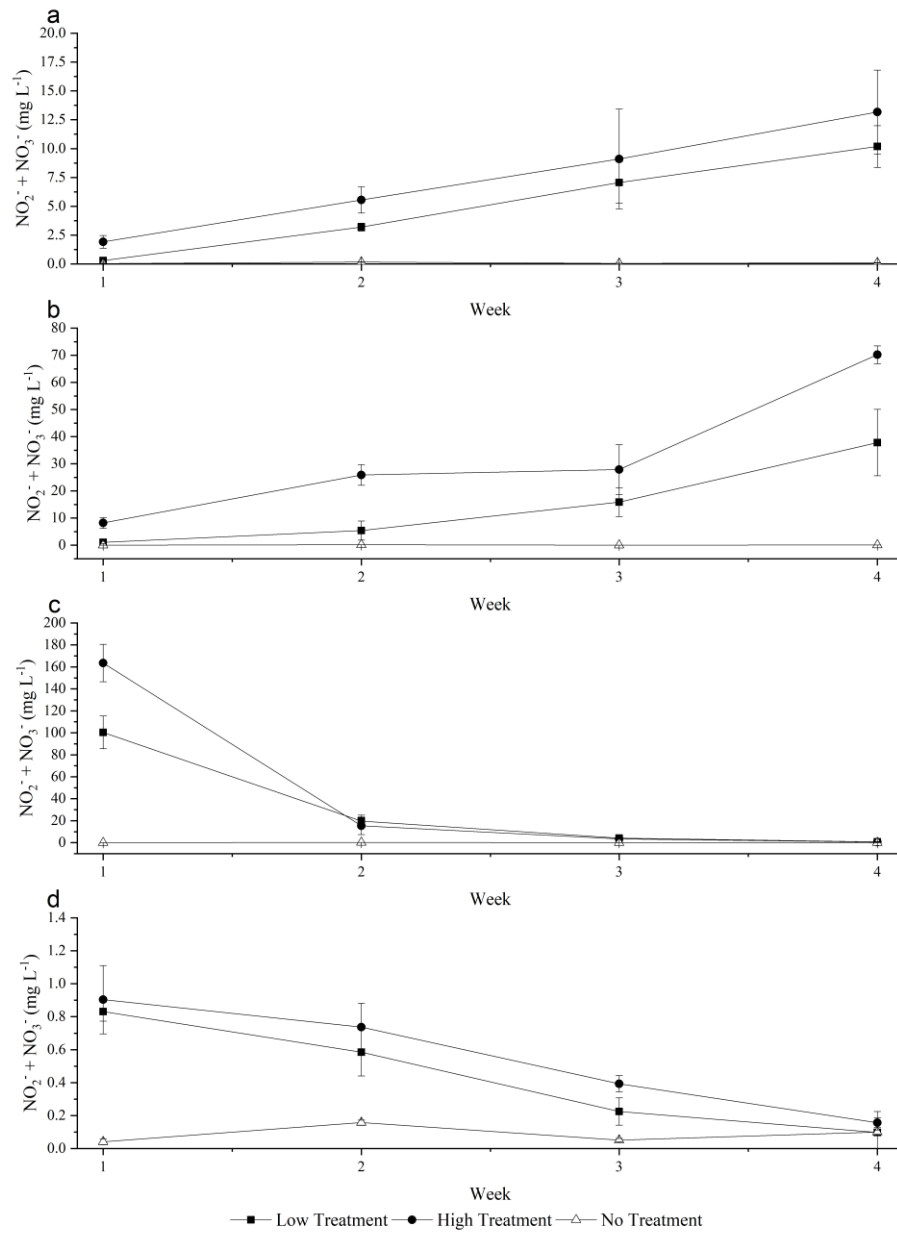


Figure 6-4. Mean weekly leachate concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N in CM (a), SM (b), GWC (c), SCG (d) from the Le Bleymard column group. n = 4

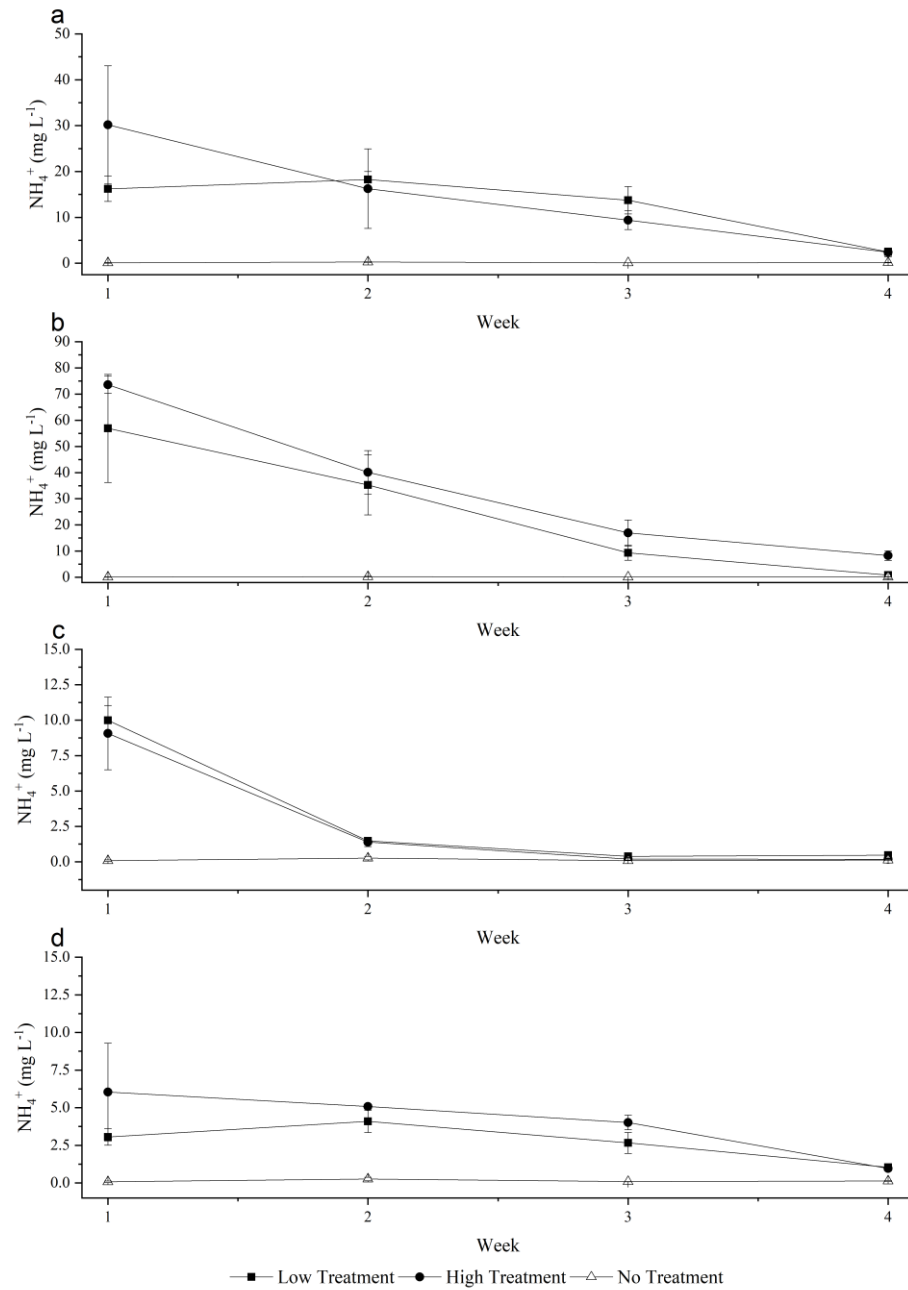


Figure 6-5. Mean weekly leachate concentrations of NH_4^+ -N in CM (a), SM (b), GWC (c) and SCG (d) from the Le Bleymard column group. n = 4

6.4.2.2 Weekly N leaching in the Tyndrum Group

Leachate concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N derived from the Tyndrum tailing material (Figure 6-6) were found to be particularly elevated after treatment with GWC (Figure 6-6c) and SM (Figure 6-7b) respectively as compared to other organic amendment treatments and the un-amended tailings ($p = >0.05$).

For both high and low applications of GWC, leachate $\text{NO}_2^- + \text{NO}_3^-$ -N concentrations were initially increased by approximately 200 mg L^{-1} before a consistent decline over the four-week period (Figure 6-6c). The high application of GWC generally elicited significantly greater leachate concentrations than the low application over the four-week period ($p = 0.006$). In contrast, leachate NH_4^+ -N concentrations following GWC treatment were substantially lower as compared with those for the other treatments (Figure 6-7c) ($p = <0.05$), although still higher than for the un-amended tailings for both high and low GWC additions ($p = <0.05$).

Application of SM promoted an immediate and consistent increase in NH_4^+ -N in the leachate (Figure 6-7d), with the higher application rate promoting an increase of $79 (\pm 7.8) \text{ mg L}^{-1}$ ($p = 0.02$) as compared to the low application. Compared to the un-amended tailings, SM increased NH_4^+ -N concentrations by $160 (\pm 22) \text{ mg L}^{-1}$ and $81 (\pm 15) \text{ mg L}^{-1}$ in the high and low applications ($p = <0.001$), indicating a substantial increase in tailing solution NH_4^+ -N. Contrastingly, leachate concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N for SM treated mining material were below the limits of detection ($<0.01 \text{ mg L}^{-1}$) throughout the experimental period (Figure 6-6b).

Leachate concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N following treatment with CM were found to be highly variable (Figure 6-6a), with only the concentrations in leachate collected from the high application rate-columns in week three showing significant differences from those in leachates from the un-amended tailings. Ammonium concentrations in leachates from the CM-treated tailing material were significantly higher than those in the leachates from un-amended tailings throughout the experimental period (Figure 6-7a) ($p = 0.008$). Cattle manure

treatment increased $\text{NH}_4^+\text{-N}$ concentration in the leachate by $6.1 (\pm 1.8)$ and $4.8 (\pm 3.2) \text{ mg L}^{-1}$ in the high and low treatments respectively, with increasing applications generally promoting greater concentrations of $\text{NH}_4^+\text{-N}$ in the leachate (Figure 6-7a).

The application of SCG led to an erratic leaching profile for both $\text{NO}_2^- + \text{NO}_3^- \text{-N}$ and $\text{NH}_4^+\text{-N}$ (Figure 6-6d and Figure 6-7d). Leaching of both forms of N significantly increased in week two, resulting in significantly elevated concentrations compared with those in leachates from un-amended tailing material ($p = <0.05$), before declining in week three. Ammonium was found to be the dominant form of available N in the leachate from SCG-amended material, with significantly higher concentrations throughout the experiment as compared with those for the un-amended tailings ($p = 0.04$). Mean weekly increases in $\text{NH}_4^+\text{-N}$ were $3.2 (\pm 1.1)$ and $1.9 (\pm 0.3) \text{ mg L}^{-1}$ for low and high application rates respectively.

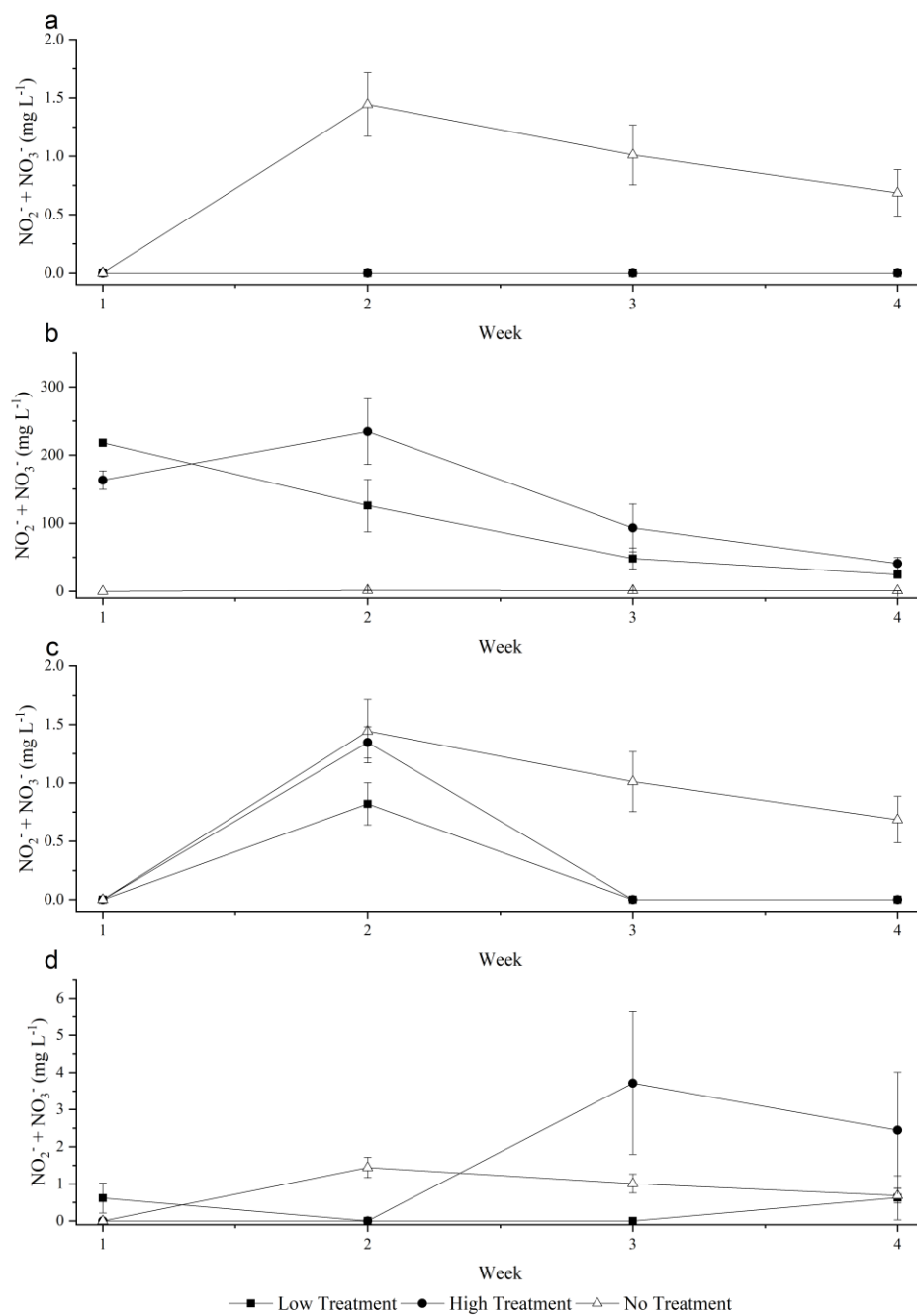


Figure 6-6. Mean weekly leachate concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N in CM (a), SM (b), GWC (c), SCG (d) from the Tyndrum column group. $n = 4$

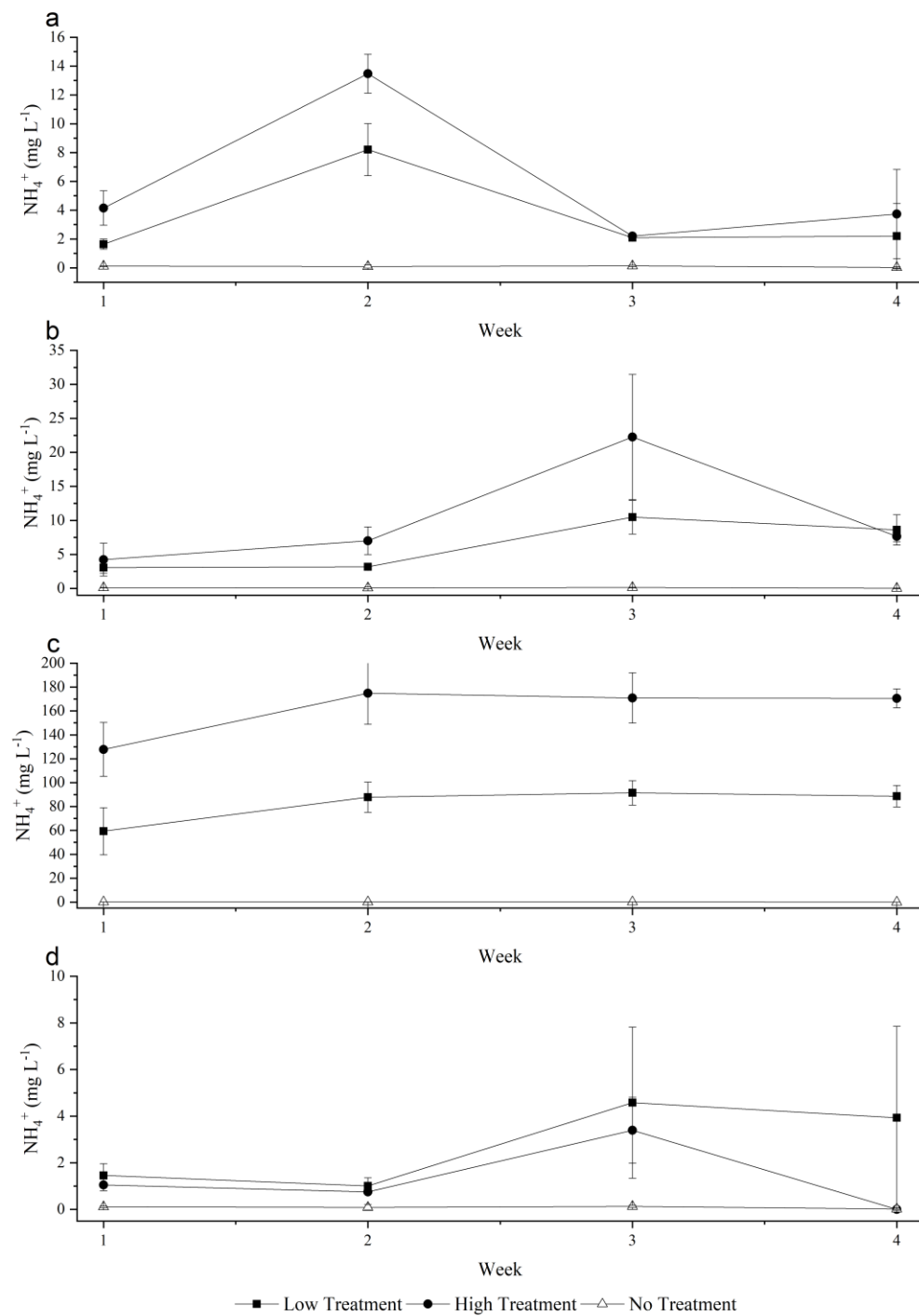


Figure 6-7. Mean weekly leachate concentrations of NH_4^+ -N in CM (a), SM (b), GWC (c) and spent coffee grounds (d) from the Tyndrum column group. n = 4

6.4.2.3 Weekly P leaching in the Le Bleymard and Tyndrum Columns

Concentrations of P in the collected leachate over the experimental period for both mine sites are displayed in Figure 6-8 and Figure 6-9.

For the Tyndrum mine tailings material, amendment with CM and SM (Figure 6-8a and b) promoted the higher leachate P concentrations compared with the other amendments and the un-amended material ($p = <0.05$), with associated increases of P with higher application rates ($p = <0.05$). Amendment with SM increased mean weekly leachate P concentrations by $33 (\pm 3.3)$ and $14 (\pm 1.2)$ mg L^{-1} in the high and low treatments, respectively, compared to the un-amended tailings, whereas a slightly smaller increase was found for corresponding CM treatments ($8.6 (\pm 1.7)$ and $2.8 (\pm 0.8)$ mg L^{-1}). In particular, SM treatments were found to give the highest leachate concentrations of P in the leachate compared with all other treatments and the un-amended tailings ($p = <0.05$). Both SM and CM amendments promoted a stable leaching trend of P over the experimental period, with a slight upward trend in the tailings containing CM (Figure 6-8a).

Both GWC and SCG amendments of the Tyndrum mine tailing material led to increased leachate P concentrations, with high and low application of GWC producing a weekly increase of $0.17 (\pm 0.06)$ and $0.09 (\pm 0.02)$ mg L^{-1} and equivalent applications of SCG eliciting an increase of $0.62 (\pm 0.6)$ and $0.3 (\pm 0.14)$ mg L^{-1} ($p = <0.05$). Leachate P concentrations for the GWC amendment were consistent with the exception of week 2 (Figure 6-8c) whereas those for SCG amendment decreased over time (Figure 6-8d).

Overall, leachate P concentrations followed the order: SM > CM > SCG > GWC amendment of the Tyndrum mine tailing material and were higher than those for the un-amended material.

For the Le Bleymard mine tailing material, SM and SM treatment generated the highest P leachate concentrations, with higher application rates promoting greater P release ($p = < 0.05$) (Figure 6-9a and b). The mean weekly P concentrations in leachates from the SM-amended material were higher than those for the un-amended tailings by $4.1 (\pm 2.1)$ and $1.2 (\pm 1.4)$ mg L^{-1} for the high and low applications, respectively. CM-amendment resulted in an

equivalent increase of $1.5 (\pm 0.9)$ and $0.2 (\pm 0.2)$ mg L⁻¹ when compared with the un-amended tailings. A significant decrease in leachate P presence concentrations was observed for all application rates for both CM and SM ($p = <0.001$), no significant differences between low application rates of CM and SM and the un-amended tailings by week 4 ($p = >0.05$). Mean weekly high and low applications of SM induced significantly higher leachate P than the corresponding rates in CM ($p = < 0.05$).

Green waste compost amendments resulted in an erratic concentration of P in the leachate, with a significant increase observed in weeks two and three before a decline to below detection limits in week four ($p = <0.05$) (Figure 6-9c). Amendment with SCG was observed to significantly increase mean weekly leachate P by $0.6 (\pm 0.1)$ and $0.9 (\pm 0.06)$ mg L⁻¹ for the high and low applications rates, respectively ($p = <0.05$), with no significant differences in P concentrations over time ($p = >0.05$) (Figure 6-9d).

In all replicates across the experimental period the un-amended tailings had as expected, a leachate concentration of P less than the limit of detection ($7.9 \mu\text{g L}^{-1}$).

Overall, leachate P concentrations for the Le Bleymard tailings followed the same order identified for the Tyndrum material (SM > CM > SCG > GWC) and again these were higher than the concentrations obtained by leaching the un-amended material.

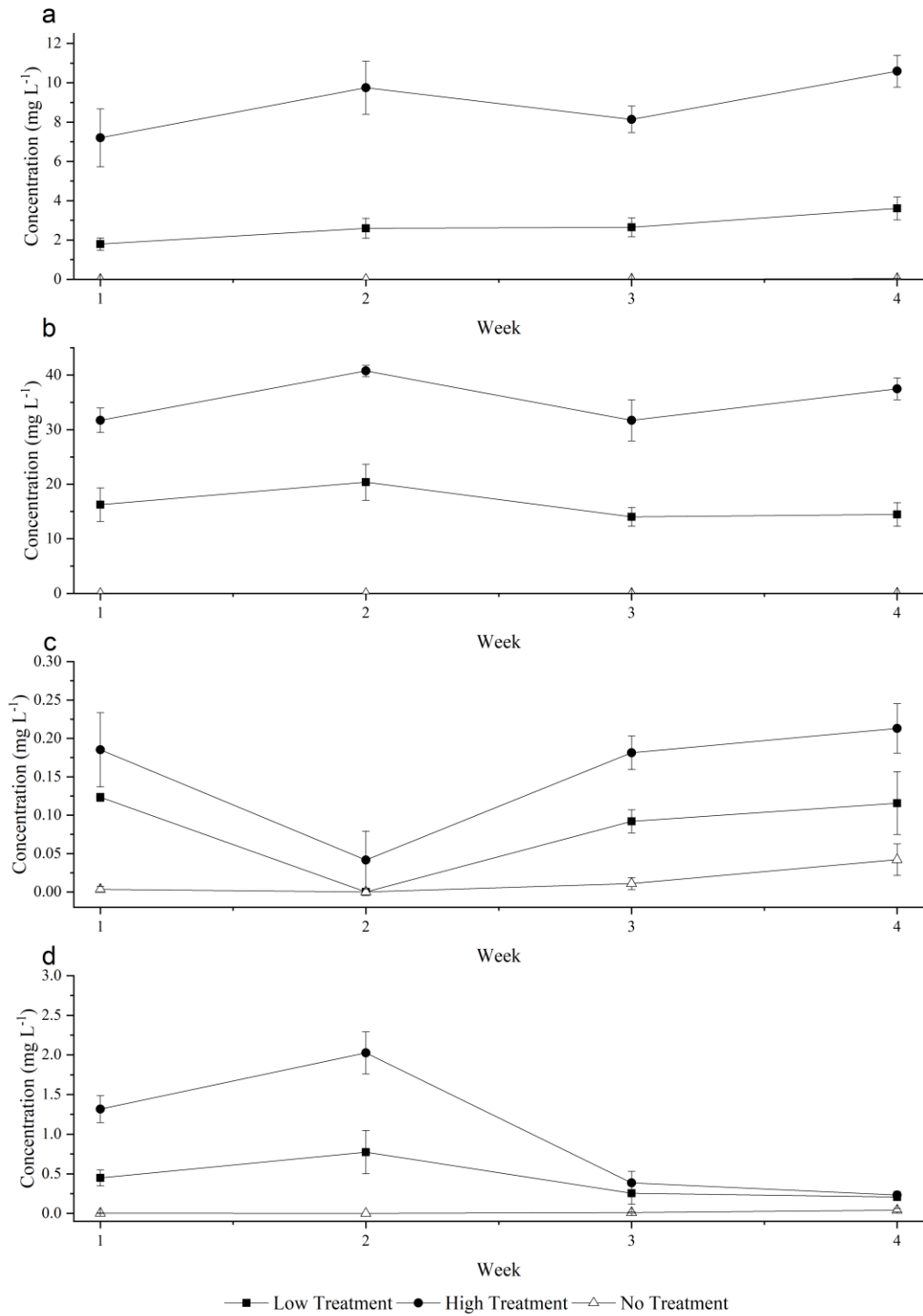


Figure 6-8. Mean weekly leachate concentrations of P in CM (a), SM (b), GWC (c), SCG (d) in the amended Tyndrum group. n = 4.

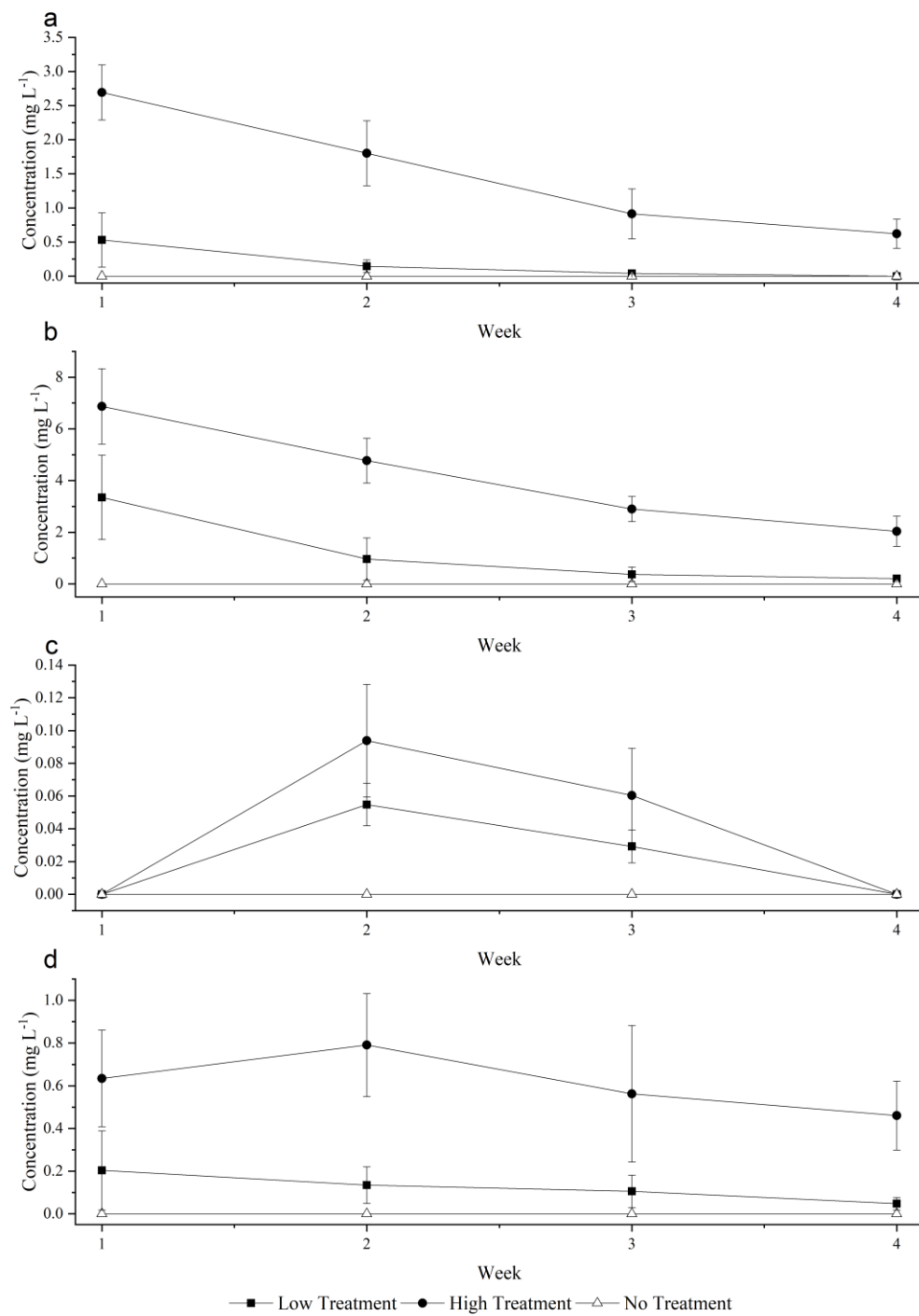


Figure 6-9. Mean weekly leachate concentrations of P in CM (a), SM (b), GWC (c), SCG (d) in the amended Le Bleymard group. n = 4.

6.4.2.4 Comparison of Leachate Concentrations of P between Le Bleymard and Tyndrum Tailings.

Mean P concentrations in leachate for each treatment group and application are presented in (Figure 6-10). Mean concentrations were found to be significantly higher for the CM and SM treatments of the Tyndrum mine tailing material as compared with those for the Le Bleymard material ($p = <0.05$). High and low applications of SM applied to the Tyndrum tailings increased mean weekly leachate concentrations of P by $31.3 (\pm 5.2)$ and $15.1 (\pm 3.7)$ mg L^{-1} , respectively, as compared with those for the Le Bleymard material, with similar results for CM ($7.4 (\pm 2.2)$ and $2.5 (\pm 0.9)$ mg L^{-1} for high and low treatments, respectively). No significant between-site differences were found for the results of GWC or SCG amendments ($p = >0.05$).

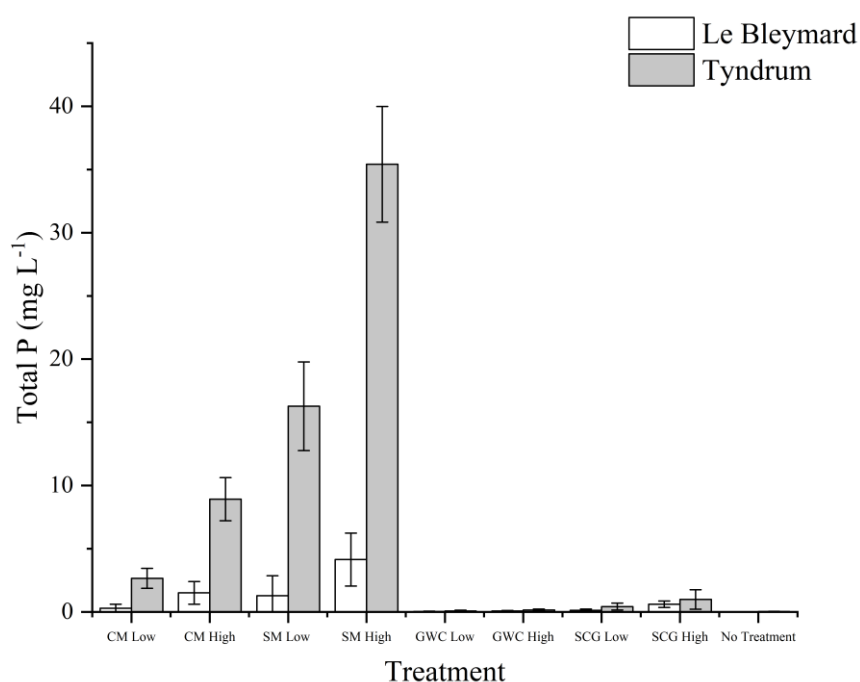


Figure 6-10. Mean P leachate concentration for each treatment over the four-week period in the tailings sourced from Tyndrum and Le Bleymard. CM = Cattle Manure, SM = Sheep Manure, GWC = Green waste compost, SCG = Spent coffee grounds.

6.4.2.5 Water soluble P in the Solid Phase

Concentrations of water-soluble P extracted from the solid phase of the amended and un-amended tailings for both Tyndrum and Le Bleymard are presented in Table 6-2.

Sheep manure amendment led to the highest concentrations of water-soluble P extracted from the post-leaching solid phase material from both sites, with increasing application rates producing a proportional increase in concentration. Cattle manure amendment had a greater impact than GWC amendment on water soluble P concentrations for the Tyndrum mine tailings material, with the opposite effect noted in the Le Bleymard tailings. Spent coffee grounds had the smallest impact on water soluble P as compared with the other amendments, and the low application rate found to be statistically similar to the control in the Le Bleymard amended tailings.

Table 6-2. Concentrations of water-soluble P extracted from the solid phase of the treated and un-amended tailings from Tyndrum and Le Bleymard. Means that do not share the same letter are significantly different for each tailing group ($\alpha = 0.05$).

Group		Le Bleymard (mg kg ⁻¹)	Tyndrum (mg kg ⁻¹)
CM	Low	31 (± 1.5) ^e	25 (± 2.6) ^d
	High	38 (± 1.7) ^d	68 (± 12) ^c
SM	Low	59 (± 5.5) ^b	166 (± 29) ^b
	High	106 (± 2.3) ^a	256 (± 18) ^a
COM	Low	45 (± 1.9) ^c	15 (± 1.2) ^e
	High	63 (± 3.9) ^b	29 (± 2.3) ^d
SCG	Low	22 (± 1.5) ^f	5.3 (± 0.54) ^g
	High	28 (± 1.5) ^e	8.1 (± 0.47) ^f
No Treatment	-	21 (± 2.3) ^f	1.4 (± 0.3) ^h

Comparing treatments across both sites, water soluble P concentrations after applications of SM were significantly higher for the Tyndrum tailings as compared to those for the Le Bleymard tailing material ($p = < 0.001$). If the high

baseline for the un-amended tailings in the Le Bleymard samples is accounted for then the addition of water-soluble P from organic amendments in the Tyndrum samples were generally significantly higher compared to the Le Bleymard group, with the exception of GWC treatments ($p = <0.001$).

6.4.3 Comparison of Redox Conditions in the Leachate between Le Bleymard and Tyndrum Tailings.

The redox potential (Eh) of the collected leachate in the Tyndrum and Le Bleymard tailings were found to be consistently between 300 and 500 mV for both sites (Appendix 2). There were no significant differences observed between treatment groups or between the Tyndrum and Le Bleymard tailings ($p = >0.05$). Conditions were therefore considered to be aerobic throughout the experimental conditions for all treatments (Bleam 2016).

6.4.4 Comparison of Leachate pH between Le Bleymard and Tyndrum Tailings.

The geometric mean of pH (due to pH following a logarithmic scale) for each organic amendment treatment in the Tyndrum and Le Bleymard amended tailing material is presented in Figure 6-11 and Figure 6-12 respectively.

For the amended mine tailings from Tyndrum, all applications of organic amendments significantly increased pH as compared to the un-amended tailings over the experimental period ($p = < 0.001$). Cattle and SM amendments (Figure 6-11a and b) were observed to promote consistently higher soil leachate pH as compared with GWC and SCG amendments ($p = < 0.05$), with high applications of SM resulting in significantly greater increases of leachate pH than other amendments or application rates. The pH of the leachate was found to be strongly correlated with leachate $\text{NH}_4^+\text{-N}$ ($R^2 = 0.67$) concentrations, which were particularly elevated in SM and CM treatments (section 6.4.2.1-2). In general, increasing applications of SM and CM promoted higher leachate pH, with the exception of week four, where a decline in pH was observed.

Although GWC and SCG treatments were not as effective at raising pH as compared to the Cm and SM treatments ($p = <0.05$), both amendments increased

pH significantly compared with the un-amended tailings (Figure 6-11c and d). A significant increase in pH for both GWC and SCG treatments was observed in week 2 ($p = < 0.001$), thereafter a consistent pH in the collected leachate was observed.

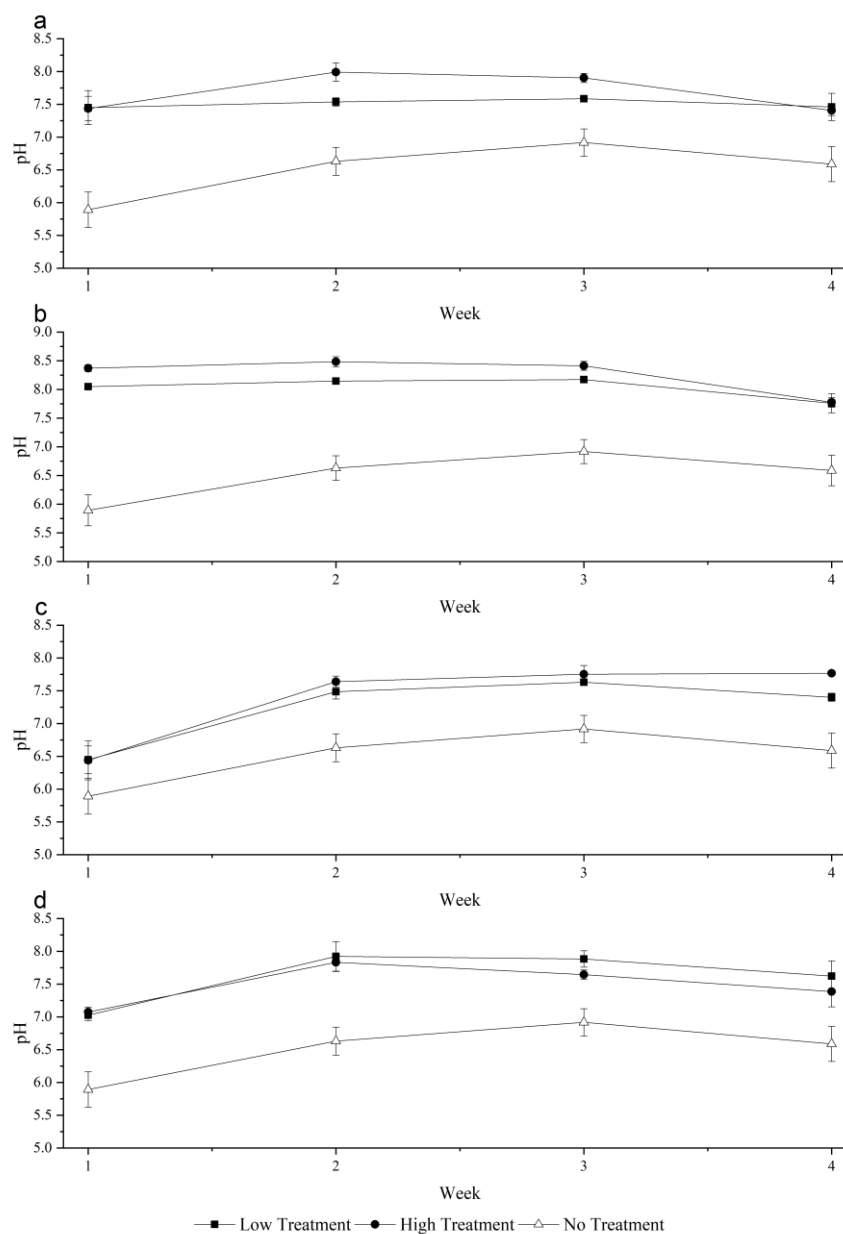


Figure 6-11. Geometric mean leachate pH in CM (a), SM (b), GWC (c), SCG (d) in the amended Tyndrum group. $n = 4$.

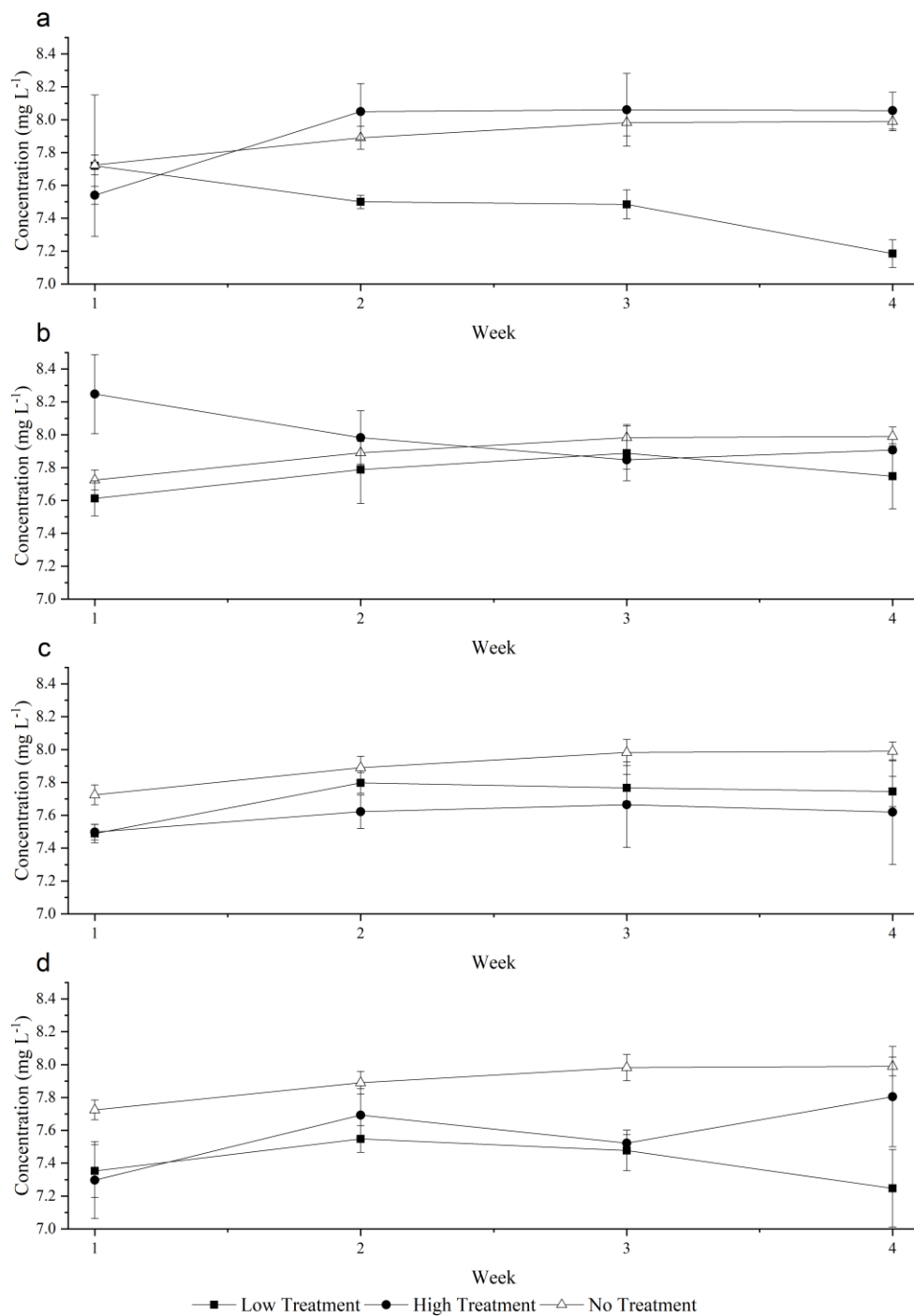


Figure 6-12. Geometric mean leachate pH in CM (a), SM (b), GWC (c), SCG (d) in the amended Le Bleymard group. n = 4.

For the Le Bleymard mine tailing material (Figure 6-12a – d), most of the organic amendments material did not affect leachate pH to a significant extent. The results for the high application of CM (Figure 6-12a) were not significantly

different from those for the un-amended tailings over time ($p = 0.19$). In contrast the low application rate decreased the leachate pH over time ($p = <0.001$). A similar effect was observed following the SM treatment, where the high application promoted a slight decrease with time from an initially elevated pH as compared to the low and un-amended tailings ($p = 0.002$). The low application of SM did not significantly change the leachate pH when compared to the un-amended tailings over time ($p = 0.72$).

High and low applications of GWC resulted in a lowering of leachate pH in weeks one and two as compared to the un-amended tailings (Figure 6-12c) ($p = <0.001$), with pH not found to be significantly different to either the low application rate or the un-amended tailing material for the rest of the experimental period ($p = 0.23$). The high application of SCG were observed to lower the leachate pH of the tailings as compared to the un-amended columns throughout the experiment ($p = <0.001$) (Figure 6-12d), whereas the pH in tailings amended with the low application rate of SCG were only significantly different in weeks one and three as compared to the un-amended tailings ($p = <0.05$). No significant differences in pH were observed between low and high SCG application rates ($p = 0.2$) throughout the experiment.

Comparing the two sites (Figure 6-13), the pH of un-amended tailings was significantly higher in leachates from the Le Bleymard mine tailing material as compared with those from the Tyndrum material ($p = <0.001$). High applications of SM increased mean leachate pH as compared to GWC treatments for tailing material from both sites ($p = 0.004$), with no other significant differences observed between groups ($p = >0.05$).

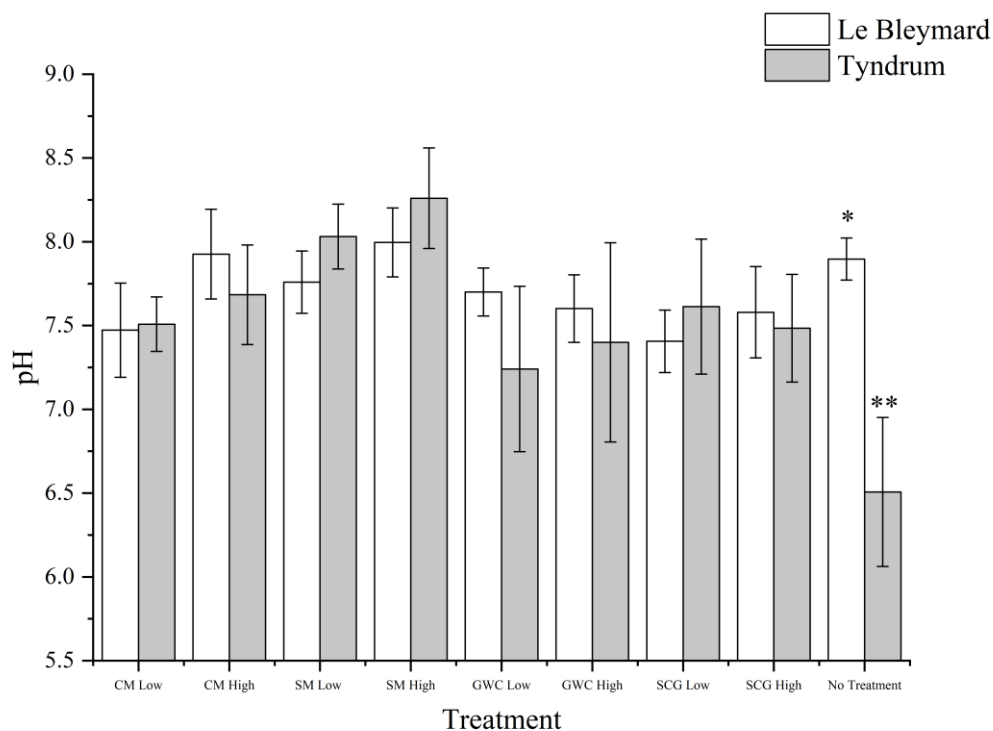


Figure 6-13. Geometric mean of pH for all treatments and un-amended tailings for Le Bleymard and Tyndrum groups.

6.4.5 Total Nitrogen (TN), Extractable $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N Concentrations in Post-leaching Column Solid Phase Mine Tailing Material from Tyndrum

Results concerning TN, extractable $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N are presented in (Table 6-3). The high application rate of SCG produced the greatest increase in solid phase TN as compared with all other organic amendments and application rates. The lower application of SCG also lead to significantly higher solid phase TN than CM and GWC treatments. All incorporations of organic amendments increased solid phase TN with the exception of CM and the low application of GWC (Table 6-3).

Table 6-3. Total N, NO₂⁻ + NO₃⁻-N and NH₄⁺-N of the solid phase material of the Tyndrum amended tailings. Means that do not share the same letter are significantly different for each analyte ($\alpha = 0.05$).

Group		TN (%)	NO ₂ ⁻ + NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)
CM	Low	0.04 (± 0.01) ^{de}	1.2 (± 0.35) ^d	3.3 (± 0.6) ^{ab}
	High	0.06 (± 0.02) ^{cde}	2.8 (± 0.56) ^c	5.2 (± 1.3) ^b
SM	Low	0.07 (± 0.03) ^{bcd}	23.7 (± 9.3) ^b	16.1 (± 9.3) ^a
	High	0.09 (± 0.01) ^{bc}	59.4 (± 11.9) ^a	13.7 (± 7.9) ^a
COM	Low	0.05 (± 0.004) ^{de}	3.7 (± 0.36) ^c	0.6 (± 0.11) ^{cd}
	High	0.07 (± 0.008) ^{cd}	4.5 (± 0.66) ^c	0.4 (± 0.03) ^{cd}
SCG	Low	0.10 (± 0.02) ^a	0.07 (± 0.02) ^f	0.72 (± 0.09) ^c
	High	0.15 (± 0.03) ^b	0.08 (± 0.01) ^f	1.1 (± 0.03) ^c
Control	-	0.02 (± 0.002) ^e	0.26 (± 0.01) ^e	0.2 (± 0.04) ^d

Concentrations of extractable NO₂⁻ + NO₃⁻-N were highest in the tailings amended with SM, with greater application rates producing higher concentrations. All other organic amendments produced significantly higher concentrations of NO₂⁻ + NO₃⁻-N as compared to the un-amended tailings, with the exception of SCG treatments where concentrations were significantly lower.

Extractable NH₄⁺-N was highest for the SM-amended material, with no significant differences between the results for the two application rates. The extractable concentrations for the CM and SCG-amended tailing material were also significantly higher as compared with those for the un-amended control. Green waste compost treatments were not significantly different in extractable NH₄⁺-N as compared to the un-amended tailings.

6.4.6 Total Nitrogen, NO₂⁻ + NO₃⁻-N and NH₄⁺-N Concentrations in Amended Le Bleymard Tailings

Concentrations of TN, and extractable NO₂⁻ + NO₃⁻-N and NH₄⁺-N are displayed in Table 6-4. Solid phase TN was found to be significantly higher in all of the organic amendment treated mine tailing materials as compared with the

untreated control, with the highest concentration of TN resulting from the high application of SCG.

Extractable $\text{NO}_2^- + \text{NO}_3^-$ -N was highest for the SM treated tailings, followed by those treated with GWC and then CM. Again, higher application rates generally increased the concentrations of this form of N. Compared to the un-amended control, SCG application led to lower extractable $\text{NO}_2^- + \text{NO}_3^-$ -N. Extractable NH_4^+ -N concentrations were below limits of detection for GWC treatments and low application rates of CM, with only high application rates of SM demonstrating significant differences in concentrations of extractable NH_4^+ -N to the CM and GWC amended tailings.

Comparing the forms of mineral N demonstrated that, with the exception of SCG where no statistical differences were observed, there was significantly greater extractable $\text{NO}_2^- + \text{NO}_3^-$ as compared to NH_4^+ in the solid phase ($p = <0.05$).

Table 6-4. Total N, $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N extractable N of the solid phase material of the Le Bleyard amended tailings. Means that do not share the same letter are significantly different for each analyte ($\alpha = 0.05$).

Group		TN (%)	$\text{NO}_2^- + \text{NO}_3^-$ (mg kg ⁻¹)	NH_4^+ (mg kg ⁻¹)
CM	Low	0.06 (± 0.01) ^d	0.88 (± 0.02) ^d	<0.01
	High	0.08 (± 0.003) ^{cd}	10.5 (± 6.2) ^{bc}	0.04 (± 0.02) ^b
SM	Low	0.08 (± 0.007) ^{cd}	32.1 (± 4.0) ^{ab}	0.06 (± 0.03) ^{ab}
	High	0.11 (± 0.006) ^b	45.6 (± 10.1) ^a	0.7 (± 0.2) ^a
COM	Low	0.06 (± 0.01) ^d	9.4 (± 0.3) ^{bc}	<0.01
	High	0.08 (± 0.005) ^d	9.8 (± 4.7) ^{bc}	<0.01
SCG	Low	0.11 (± 0.07) ^{bc}	0.09 (± 0.03) ^e	0.12 (± 0.05) ^{ab}
	High	0.18 (± 0.03) ^a	0.21 (± 0.3) ^e	0.13 (± 0.1) ^{ab}
Control	-	0.03 (± 0.005) ^e	4.1 (± 2.0) ^{cd}	0.1 (± 0.08) ^{ab}

6.4.7 Total Carbon and Microbial Biomass Carbon in Amended Tyndrum Tailings

Total C in the tailings sourced from Tyndrum was significantly elevated in the tailings which had the high application rates of SCG as compared to all other treatments (Table 6-5). Low applications of SCG were found to increase the TC content of the mine tailing material as compared to high and low applications of SM and CM, and the low application of GWC. Overall, all incorporations of organic material significantly increased TC in the tailings as compared to the un-amended tailing material, with the exception of low application rates of CM.

Table 6-5. Total carbon and microbial biomass carbon of the solid phase material of the Tyndrum amended tailings. Means that do not share the same letter are significantly different for each analyte ($\alpha = 0.05$).

Group		TC (%)	Microbial Biomass C ($\mu\text{g g}^{-1}$)
CM	Low	0.99 (± 0.33) ^{de}	9.9 (± 1.1) ^b
	High	1.46 (± 0.43) ^{cd}	40.6 (± 9.4)^a
SM	Low	1.18 (± 0.37) ^d	40.0 (± 23.7)^a
	High	1.51 (± 0.15) ^{cd}	62.1 (± 22.6)^a
COM	Low	1.45 (± 0.16) ^{cd}	1.0 (± 0.3) ^d
	High	1.99 (± 0.15) ^{bc}	4.0 (± 1.2) ^{bc}
SCG	Low	2.29 (± 0.43) ^b	30.0 (± 10.4)^a
	High	3.88 (± 0.47)^a	40.8 (± 13.0)^a
Control	-	0.29 (± 0.003) ^e	1.57 (± 0.3) ^{cd}

Microbial biomass carbon (MBC) was significantly increased in mine tailing material which had been amended with SM and CM and with SCG. Values were up to 60.4 $\mu\text{g g}^{-1}$ greater than that for the un-amended control (Table 6-5). Neither application rate of GWC to the tailing material promoted a significant difference in MBC as compared to the un-amended control.

6.4.8 Total Carbon and Microbial Biomass Carbon in Amended Le Bleymard Tailings

Total carbon and MBC results for the Le Bleymard columns are presented in Table 6-6. Total carbon was significantly increased upon the incorporation of all application rates of organic amendments as compared to the un-amended tailings, with the greatest increases observed for the SCG-amended mine tailing material.

Table 6-6. Total carbon and microbial biomass carbon of the solid phase material of the Le Bleymard amended tailings. Means that do not share the same letter are significantly different for each analyte ($\alpha = 0.05$).

Group		TC (%)	Microbial Biomass C ($\mu\text{g g}^{-1}$)
CM	Low	8.98 (± 0.09) ^e	17.3 (± 2.7) ^c
	High	9.35 (± 0.07) ^{de}	23.4 (± 2.3) ^{bc}
SM	Low	9.27 (± 0.08) ^{de}	29.3 (± 4.8) ^{bc}
	High	9.57 (± 0.09) ^{cd}	37.6 (± 8.5) ^{ab}
COM	Low	9.48 (± 0.16) ^d	8.1 (± 1.2) ^d
	High	10.2 (± 0.1) ^b	7.3 (± 2.6) ^d
SCG	Low	9.99 (± 0.41) ^{bc}	29.8 (± 4.1) ^{bc}
	High	11.9 (± 0.3)^a	62.7 (± 11)^a
Control	-	8.3 (± 0.06) ^f	5.5 (± 3.2) ^d

In the Le Bleymard tailings MBC was significantly improved by the addition of CM, SM and SCG as compared to the un-amended material, increasing MBC by up to $32 \mu\text{g g}^{-1}$ following the high level application of SCG (Table 6-6). No significant differences were observed between either GWC application rates or the un-amended control. Addition of SCG promoted significantly greater MBC in the amended tailings than both application rates of CM and the low application of SM.

6.4.9 Comparison of TC and Microbial Biomass Carbon in the Solid Phase between Le Bleymard and Tyndrum

Concentrations of TC were found to be significantly higher in the Le Bleymard material compared to Tyndrum ($p = <0.001$). However, after accounting for the higher baseline found in the calcareous Le Bleymard tailings the relative increases between treatments across the two sites were not significantly different ($p = > 0.05$).

Microbial biomass carbon was not found to be significantly different between Tyndrum and Le Bleymard material ($p = 0.8$), with the exception of CM, where the low applications rate was found to induce significantly higher MBC in the Le Bleymard group and the opposite found for the high application. Green waste compost treatments in Le Bleymard were also found to increase MBC relative to the same application to the Tyndrum tailings ($p = <0.001$). For the mine tailing material from both sites, all amendments significantly increased MBC as compared to both GWC applications and the un-amended tailings ($p = < 0.001$), with the MBC of the un-amended tailings from Le Bleymard demonstrating significantly greater MBC than the un-amended Tyndrum tailings ($p = < 0.001$).

6.4.10 Solid Phase pH in Amended Tyndrum and Le Bleymard Tailing Material

The ranges in pH for the tailing material for Tyndrum and Le Bleymard are presented in (Table 6-7). For the Tyndrum amended tailings, pH was significantly increased upon the incorporation of all application rates of CM, SM and GWC, whereas SCG did not promote a significant difference in pH compared to the un-amended control. In general, high application rates of Cm and SM had a greater impact on pH. The pH of the tailing material was strongly correlated with $\text{NH}_4^+\text{-N}$ concentrations ($R^2 = 0.75$).

For the tailings sourced from Le Bleymard, applications of organic amendments were generally observed to decrease tailings pH, with the greatest changes observed in the high application rate of SCG. The incorporation of both GWC application rates to the tailings from Le Bleymard did not elicit a change in the pH of the amended material.

Overall, significant differences were found between sites in terms of un-amended controls, GWC treatments and low application rates of CM ($p = <0.001$). No significant differences were found in other groups between sites. In general the pH of the tailing material was significantly higher in the Le Bleymard group as compared to the tailings sourced from Tyndrum ($p = <0.05$).

Table 6-7. pH ranges in the tailing material from the Tyndrum and Le Bleymard columns. Means that do not share the same letter are significantly different for each analyte ($\alpha = 0.05$).

Group		Tyndrum	Le Bleymard
CM	Low	7.47 – 7.72 ^{bcd}	7.81 – 7.97 ^c
	High	7.82 – 7.91 ^{ab}	7.8 – 7.99 ^{bc}
SM	Low	7.74 – 8.21 ^a	7.81 – 8.04 ^{abc}
	High	7.64 – 8.11 ^{ab}	7.55 – 7.65 ^d
COM	Low	7.22 – 7.51 ^{cd}	8.0 – 8.06 ^{ab}
	High	7.58 – 7.65 ^{bc}	7.89 – 8.02 ^{abc}
SCG	Low	7.25 – 7.32 ^{de}	7.66 – 7.78 ^d
	High	7.25 – 7.33 ^{de}	7.31 – 7.49 ^e
Control	-	6.9 – 7.25 ^e	8.03 – 8.07 ^a

6.5 Discussion of Tailing Nutrient and Microbial Findings

6.5.1 Tyndrum Tailing pH

Amendments promoted an increase in both the leachate and the post leaching solid phase pH, with CM and SM additions leading to an appreciably higher pH compared to the SCG and GWC treatments. Cattle manure and SM were found to have significantly elevated solid phase and leachate pH values in comparison to the un-amended control, GWC and SCG, an effect that was strongly linked to increasing $\text{NH}_4^+\text{-N}$ in the solid phase and leachate solution, with the presence of $\text{NH}_4^+\text{-N}$ contributing to increasingly alkaline conditions in these treatment groups (Yan *et al.* 1996). Increasing concentrations of Mg^{2+} and Ca^{2+} were observed in the leachate of all amended tailing materials compared to the un-amended tailings (Figure 6-20) (Section 6.6.4), possibly indicating that large introductions of base cations increased the buffering capacity of the amended

tailings along with bicarbonates and the absorption of H^+ , from other constituents that are likely present in the amendments (Olsen *et al.* 1970; Wong *et al.* 1998; Whalen *et al.* 2000; Walker *et al.* 2004; Farrell and Jones 2010). The initial elevation in pH is likely due to the substantial introduction of the above properties to an otherwise sandy, skeletal system, improving the buffering capabilities and chemical complexity of the tailing material (Yan *et al.* 1996; Murphy 2015).

However, despite an initial increase in pH, the permanency of this effect requires consideration. Concentrations of Mg^{2+} and Ca^{2+} showed a strong decline in concentration in the leachate from GWC and SCG-treated tailings, demonstrating a loss of base cations from the system and a replacement by NH_4^+ or H^+ (Bache 1984; Jansen van Rensburg *et al.* 2009; Goulding 2016). This suggests that in the longer term there may be net acidification of the amended tailings as constituents for buffering are diminished via leaching effects. This occurrence may be suggested by pH leachate data in week four of the SCG, where initially elevated pH appears to be reducing slightly, and is reinforced by the strong relationship between Mg^{2+}/Ca^{2+} and pH observed in SM and CM.

Despite the initial increases in pH from CM and SM, significant acidification of tailings amended with these manures may occur in the longer term due to nitrification resulting from elevated NH_4^+ -N concentrations observed in these treatments. High inputs of N in the form of NH_4^+ are well known to initially increase soil pH (Anthonisen *et al.* 1976; Haynes 1986). However, the process of nitrification would likely contribute to a net acidification over time following the release of protons into the tailing environment as demonstrated by the oxidation of ammonia to nitrite ($NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$) and subsequently the oxidation of nitrite to nitrate ($NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$). Therefore, as microbial processes occur over time, it is possible that the incorporation of CM, and, in particular, SM may have significant impacts on the pH of the amended tailings due to the elevated ammonium concentrations in this organic amendment.

The acidification of the tailing material could have substantial impacts on nutrient availability, and critically, PTE behaviour. PTE results are discussed in detail in section 6.6.

6.5.2 Le Bleymard pH

In contrast, the carbonate rich tailings sourced from Le Bleymard had a significantly higher baseline pH, as reflected in the alkaline nature of the un-amended leachate and tailings. The reduction of pH observed in the weekly leachate and the solid phase sampling results demonstrated an acidification of the tailing material under some amendment applications, although the tailings remained neutral to alkaline upon the conclusion of the experiment.

The acidification of the tailing material could be attributed to the introduction of organic acids, increasing the presence of H^+ and therefore reducing pH (Caravaca *et al.* 2005; Santibáñez *et al.* 2008; Dede *et al.* 2017). The inclusion of basic cations from the organic amendments was not significantly higher as compared to the un-amended tailings, due to their high inherent concentration of Ca^{2+} throughout the leaching period and so an introduction of Mg^{2+}/Ca^{2+} was not likely to have a significant impact on pH. Conversely, evidence for nitrification is apparent in the leaching characteristics and in the comparisons of $NO_2^- + NO_3^-$ - N and NH_4^+ - N in the solid phase at the end of the experiment, which may be a factor in the reduction of pH for CM and SM in particular due to the resulting release of H^+ during nitrification.

The high buffering capacity of the Le Bleymard tailings may have differing impacts in terms of PTE immobilisation and nutrient availability. PTE solubility will likely be minimised by the formation of immobile hydroxide species (e.g. $Pb(OH)_2$, $Zn(OH)_2$ and $Cd(OH)_2$) reducing the environmental risk from these PTEs (Alloway 2012). In contrast, elevated pH may limit the availability of plant nutrients e.g. (Zn, Cu and P) by the formation of immobile phases and induce N loss via increased NO_3^- . Furthermore, plant stress is often increased under highly alkaline conditions, primarily via cellular damage and oxidative stress in roots (Zhang *et al.* 2017). Therefore, the alkaline potential of the Le Bleymard tailings may make re-vegetation challenging.

Overall, it is unknown whether the loss of basic cations and introduction of soluble organic acids will be limited by the increase in chemical complexity and apparent buffering capacity of the tailing material when an excess of organic amendment is introduced. Extended experiments following the pH of amended tailing systems will be required to ascertain the long-term impacts of adding these amendments to the tailing material sourced from Tyndrum and Le Bleymard.

6.5.3 Nitrogen Leaching and Availability

The leaching characteristics, total concentrations and mineral forms of N across the treatments and the two tailing substrates displayed diverse trends in N availability and as a potential controlling factor for pH.

Sheep manure and CM initially introduced high concentrations of NH_4^+ into the tailing materials, elevating pH in the Tyndrum tailings and promoting a consistent release of NH_4^+ from the columns and increasing both $\text{NO}_2^- + \text{NO}_3^-$ -N and NH_4^+ -N in the solid phase. In contrast, in the Le Bleymard tailings a significant decrease of NH_4^+ -N was observed over time for both CM and SM with a concurrent increase in $\text{NO}_2^- + \text{NO}_3^-$ -N, suggesting that significant nitrification processes were taking place in the columns. The optimum functioning of *Nitrosomonas* and *Nitrobacter* at elevated pH is well established and known to be rapid in alkaline soils (Morrill and Dawson 1967; Dancer *et al.* 1973; Ward 2013). Thus, it is likely the primary cause of increasing presence of $\text{NO}_2^- + \text{NO}_3^-$ -N in the leachate over time in the Le Bleymard tailings. Furthermore, comparisons of high NH_4^+ -N and low $\text{NO}_2^- + \text{NO}_3^-$ -N in SM and CM initially compared to the inverse findings observed at the end of the experiment suggests a conversion from NH_4^+ -N to $\text{NO}_2^- + \text{NO}_3^-$ -N in the amended tailings from Le Bleymard. Similar findings were observed in the final solid phase extractions of the Tyndrum tailings, although there was no significant evidence of $\text{NO}_2^- + \text{NO}_3^-$ leaching from this group. This may be due to slower nitrification processes, which generally begin 3-4 weeks after the application of an NH_4^+ -N - rich source and might have been side of the experimental time period (Haynes 1986).

In summary, the nitrification process suggests an active microbial system in the tailings and a readily available form of N for plant and microbial uptake, improving the nutrient content of the tailing material as demonstrated in the solid phase extractions. Moreover, several authors have indicated the usefulness of incorporating materials high in $\text{NH}_4^+\text{-N}$ into calcareous soils, based on the electrostatic attraction of the NH_4^+ ion to negative surfaces generated at high pH and therefore the retention of this available form of N in the soil (Pasda *et al.* 2001; Maqsood *et al.* 2016).

Conversely, increasing concentrations of $\text{NO}_2^- + \text{NO}_3^-\text{-N}$ in the leachate suggests that significant losses can be generated by the formation of $\text{NO}_2^- + \text{NO}_3^-\text{-N}$ due to the sandy texture of the tailings, particularly at higher pH where NO_3^- mobility will be increased (Whitehead 2000; Rowell *et al.* 2001; Gilmour *et al.* 2003; Rigby *et al.* 2016). Moreover, the process of nitrification is linked to the acidification of soil systems, leading to possible risks of PTE leaching resulting from the dissolution of carbonate-held Pb and Zn in the tailings. Finally, it could also be reasoned that low $\text{NH}_4^+\text{-N}$ concentrations in the solid phase extraction may be due to high adsorption of the cation to tailing surfaces and that the 2 M L^{-1} KCl extractant did not liberate $\text{NH}_4^+\text{-N}$ into the analytical solution (Song *et al.* 2014), however extractable $\text{NO}_2^- + \text{NO}_3^-\text{-N}$ at the conclusion of the experiment was substantially higher in the organic amended tailings as compared to the control, suggesting that mineralisation had occurred.

The opposite findings in the leachate of the Tyndrum columns suggests that nitrification processes had not yet been fully established. Discharges of $\text{NH}_4^+\text{-N}$ from manures treatments were constant over time, suggesting a consistent efflux of $\text{NH}_4^+\text{-N}$ into the soil solution in the short term from a large reservoir of $\text{NH}_4^+\text{-N}$. It is possible that over time higher concentrations of $\text{NO}_2^- + \text{NO}_3^-\text{-N}$ may occur in the SM samples as $\text{NH}_4^+\text{-N}$ is mineralised into $\text{NO}_2^- + \text{NO}_3^-\text{-N}$, which may result in risks of further leaching from this treatment approach in the future. Despite the N losses from the tailings, there was a significant increase in TN, and $\text{NO}_2^- + \text{NO}_3^-\text{-N}$ in CM and SM applications, indicating a greater support for vegetation and microbial communities.

Green waste compost and SCG had differing impacts on N dynamics, with the former demonstrating a rapid loss of $\text{NO}_2^- + \text{NO}_3^-$ -N in the leachate from both sites and the latter exhibiting low leachate concentrations of NO_3^- -N. Both GWC and SCG did not appear to promote the leaching of NH_4^+ -N from either Tyndrum or Le Bleymard tailings, most likely due to the lower presence of NH_4^+ -N in these materials.

As GWC has undergone bio-degradation prior to incorporation, nitrification and mineralisation processes have already occurred, introducing a $\text{NO}_2^- + \text{NO}_3^-$ -N rich material into the tailings. The previously mentioned sandy nature of the tailings and elevated pH of the Le Bleymard tailings probably induced the leaching of $\text{NO}_2^- + \text{NO}_3^-$ -N from this treatment group and resulted in low solid phase concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N at the end of the experiment. As nitrification had already occurred in this material the potential regeneration of $\text{NO}_2^- + \text{NO}_3^-$ -N from the oxidation of NH_4^+ -N would be unlikely. Despite this, the overall concentration of plant available-N and TN was increased in GWC treatments in comparison to the control, suggesting that GWC may still provide a suitable reservoir of N to a skeletal system.

Spend coffee grounds did not promote significant leaching of $\text{NO}_2^- + \text{NO}_3^-$ -N or NH_4^+ -N from the tailings of amended Le Bleymard or Tyndrum tailings as compared with other organic amendments and interestingly resulted in lower concentrations of $\text{NO}_2^- + \text{NO}_3^-$ -N and slightly higher concentrations of NH_4^+ -N in the solid phase extractions. This phenomenon has been well-reported in literature concerning coffee waste additions to soils (Cruz *et al.* 2012; Yamane *et al.* 2014; Cruz and Marques dos Santos Cordovil 2015) and may be due to increased microbial activity of the tailings, where the wide C:N ratio of SCG produces a large input of labile C, encouraging microbial populations to rapidly expand and scavenge easily accessible N compounds to complete their metabolic cycles (Paul 2007). Although this suppression of plant-available N has been reported to last at least one year (Yamane *et al.* 2014) in agricultural settings, the long-term fate of N availability in mine tailings after SCG amendments is unknown. It is important to note that SCG produced the highest increase in TN

in both tailing materials, indicating that a large pool of conserved N remains in the tailings via SCG additions. This may be liberated over time, providing a slower release of N into the tailing substrate, which may be useful in situations where a slow release of N is required to avoid leaching of nitrates to water bodies adjacent to mine tailings (Busby *et al.* 2007).

6.5.4 Phosphorous leaching

The leaching of P from the amended tailings in comparison to the untreated material demonstrates a greater increase in mobile P which would aid in the support of vegetation development. Concentrations of P were highest in the CM and SM treatments and only moderately increased in the GWC and SCG applications. Significantly lower leachate and water soluble P concentrations were observed in the Le Bleymard tailings as compared to Tyndrum amended tailings, probably due to P fixation by Ca and the formation of insoluble calcium phosphates in the matrix of the former (Abbott and Tucker 1973; Naeem *et al.* 2013).

In general although soluble P introduction via organic materials is known to improve P availability by the retardation of P fixation (Westermann 1992; Delgado *et al.* 2002; Turner and Leytem 2004; Hopkins and Ellsworth 2005; Leytem and Westermann 2005; Sato *et al.* 2005; Garg and Kaur 2013; Audette *et al.* 2016), the decline in leached P over time suggests that fixation as $\text{Ca}_3(\text{PO}_4)_2$ or magnesium phosphate species is occurring in the Le Bleymard treatments (Turner and Leytem 2004; Hopkins and Ellsworth 2005; Al-Rohily *et al.* 2013). This indicates that over time there may be a net immobilisation and reduction in available P, despite high initial inputs. Regardless of this possible crystallisation and reduction of labile P, the overall pool of P in the tailings appears to have been significantly increased, indicating an improvement in tailing nutrient characteristics and potential support for vegetation.

6.5.6 Overall considerations for N and P

Identifying the best amendment for improving overall N and P concentrations and availability in the mine tailings tested is not immediately clear, due to the same amendments eliciting substantially different outcomes resulting from

underlying differences in tailing and organic amendment characteristics. Applications of CM, SM and GWC appear to introduce substantial concentrations of available N, which can be lost from the system via leaching. Contrastingly, SCG appears to provide a more sustainable source of N with greater retention in the tailings system. However, the low availability of N following application of SCG suggests that the SCG amended mine tailing may not support vegetation as N is sequestered in microbial biomass. For P, SM amendment results in the greatest input of labile P into the tailing system. However, this available P appears to be significantly reduced under the alkaline conditions of the Bleymard tailings.

6.5.7 Discussion: Microbial Functioning

Soil respiration has been documented as a useful indicator of the microbial functioning of a soil and can be used to infer pollutant degradation, nutrient cycling, microbial activity and response to metal pollution (Nwachukwu and Pulford 2011; Chen *et al.* 2014; Goupil and Nkongolo 2014). Therefore, the measured CO₂ fluxes can be used to infer changes in these parameters in the amended tailings from Le Bleymard and Tyndrum.

The un-amended tailings at both sites were found to have low baseline respiration rates, indicating diminished microbial functioning and the likelihood of a biotically reduced system when compared to a functioning arable soil (Beyer 1991; Kutsch and Kappen 1997). This is likely due to the low concentrations of organic C and elevated PTEs detailed in Chapter 4, reducing the capacity of the tailing material to support biological processes and complex microbial communities (Buchmann 2000; Ramsey *et al.* 2005).

The CM and SM and the SCG were found to promote a substantial increase in soil respiration, with increases of up to 1.6 mg CO₂ – C m²/d⁻¹ observed in some cases. The higher application rate of SM and SCG were generally found to increase respiration rates on both sites, suggesting improvements in soil microbial functioning. Chemical analysis of the SM and SCG show that both amendments have high OM content, labile N and reserves of immediately available and microbially accessible C (Table 6-1). The input of directly

obtainable nutrients, coupled with the inoculation of microbial communities already present in the amendments enhanced soil respiration, likely improving the cycling of nutrients and bolstering microbial communities already present in the tailing materials (Ros *et al.* 2003; Bastida *et al.* 2008; Zornoza *et al.* 2012).

Considering SM and SCG applications on both sites, addition of SM resulted in an immediate increase in respiration, followed by a stable weekly flux of CO₂, whereas SCG trends suggested a decline in respiration rate over time. Amendment with SCG led to significantly greater cold-water extractable C as compared to SM and, in the case of the Tyndrum mine tailing material, a significantly greater respiration rate in weeks two and three as compared to the SM. This finding suggests that the introduction of a concentrated and immediately accessible C source increases respiration rates initially, however, microbial consumption may be unsustainable and declines within a short period of time. Contrastingly, SM appears to promote an immediate and consistent increase in soil respiration, suggesting that this amendment can provide a more sustainable nutrient source. This is possibly due to a combination of the high C pools and considerable concentrations of NO₂⁻ + NO₃⁻ - N and NH₄⁺ - N found in the SM (Table 6-1), which provides enough nutrients for microbes to complete their life cycle and therefore sustain the respiration rate in the short term (Vinten *et al.* 2002; Silvan *et al.* 2003; Schmidt *et al.* 2011).

A similar trend is observed in the CM treatments which have similar properties to SM (Table 6-1), suggesting that these animal manures can elicit a similar effect in terms of soil respiration from the mine tailings. The evidence for CM improving microbial functioning is clear in the wider literature (Ndayegamiye and Côté 1989; de Freitas *et al.* 2003; Edmeades 2003) and it is likely that the improvements of tailing qualities are a result of the intrinsic properties of this organic amendment. Cattle manures were found to have a slightly lower respiration effect than SM however, which may be reflected in to slightly lower extractable C and NO₂⁻ + NO₃⁻ - N and NH₄⁺ - N in this material (Table 6-1).

Over both sites respiration rates from GWC treated tailings were significantly lower than all other treatments, and in the case of the Tyndrum tailings, similar

to the current fluxes found in the un-amended material. The low respiration rates observed in the GWC treatments are likely due to the stable, humified nature of this material, the higher C:N ratio (Table 6-1) and the lower concentration of cold water extractable C (CWECC) in comparison to the other amendments. Immediate C and energy sources for microbial systems are therefore likely to be diminished in these treated tailings, reducing microbial activity in comparison to the short term. It is possible however, that the incorporation of a more humified material such as GWC would have a greater long term effect than CM or SM, where the higher C:N ratios can create a slower nitrification rate, and therefore a more sustainable microbial consumption of N (Santibáñez *et al.* 2007).

The results from the respiration data support the MBC findings, where the greatest increases were observed in SCG and the lowest in the GWC treatments. This suggests that organic amendments with high concentrations of labile C can stimulate microbial populations more effectively than the more humified and stable materials such as GWC.

Comparing the effects of amendments from each site, the high application of SM was most effective when applied to the Le Bleymard and Tyndrum tailings, when accounting for tailing respiration trends over the four-week period. Cattle manure may be considered a viable alternative for similar reasons although respiration from this material was not as high as compared to SM. Green waste compost applications do not initially appear to promote respiration, suggesting that this material is more microbially inert than the others tested. Contrastingly, SCG may not be a suitable organic amendment for promoting long term soil respiration as there is evidence for rapid initial increases in respiration followed by diminishing activity in weeks 3 and 4, particularly in the experiment concerning the Tyndrum tailings.

6.5 Section Conclusions

For the Tyndrum site, the application of SCG may be the most appropriate, considering the improvements in microbial biomass, low loss of N and P, and an increase in both leachate and tailing pH. Considering the tailings from Le Bleymard the choice is more complicated. For example, the high lability of the

organic matter in the SM may counteract the precipitation effects of the calcareous tailings and the immobilisation of plant nutrients whilst bolstering microbial communities. However, the rapid nitrification of $\text{NH}_4^+\text{-N}$ and the resultant leaching of $\text{NO}_2^- + \text{NO}_3^-\text{-N}$ may simply create a loss of N in the long term, combined with acidification of carbonate held PTE. Conversely, a more inert amendment such as GWC may provide a more stable solution in the long term, despite rapid initial losses of available N. Higher applications of CM may be a viable option for this site, as losses in N and P were moderate, with only a slight acidification of the tailing material observed at the conclusion of the investigation.

Considering the respiration trends, pH, N and P leaching and N speciation in the aqueous and solid phase it is clear that different organic amendments cause diverse chemical and biological changes in the mine tailings tested, which are inherently contrasting in themselves. From this it is obvious that the generalisation on the usefulness of organic materials to increase the fertility and functioning of mine tailings is not appropriate and must be considered on a site to site basis. Despite this, some broad conclusions can be drawn:

1. The use of $\text{NH}_4^+\text{-N}$ rich organic materials on calcareous tailings will likely induce rapid nitrification, acidification and the loss of N as $\text{NO}_2^- + \text{NO}_3^-\text{-N}$, alongside possible release of PTEs.
2. Immobilisation and loss of plant nutrients is more likely to be encountered on calcareous mine tailings, presenting difficulties in improving soil conditions for revegetative purposes.
3. Amendments with higher C:N ratios may be more suitable for the remediation of mine tailings, by immediately improving microbial populations and minimising losses of N and releasing N more slowly, but high C:N ratios may also give poor vegetation performance as a result of microorganisms sequestering N.

6.6 Leaching of Potentially Toxic Elements

6.6.1 Cd Leaching: Tyndrum and Le Bleymard

For the Tyndrum mine tailing material the incorporation of all amendment types significantly reduced leachate concentrations of Cd during the experimental period (Figure 6-14a-d).

In general the reduction of Cd in the tailing leachate was not found to be significantly different between treatment types and application rates, with the exception of the high application rate of CM and both SCG treatments ($p = <0.05$) in week one (Figure 6-14(a), (d)), which were $\sim 1000 \mu\text{g L}^{-1}$ higher than SM or GWC treatments. Leachate Cd concentrations in the GWC and SCG treatments decreased significantly over time, from $80 (\pm 10)$ to $10 (\pm 1) \mu\text{g L}^{-1}$ and $150 (\pm 20)$ to $20 (\pm 6) \mu\text{g L}^{-1}$ respectively (Figure 6-14 c-d). A slight increase in Cd was observed in week four for CM and SM, however the difference was $10 \mu\text{g L}^{-1}$ for both.

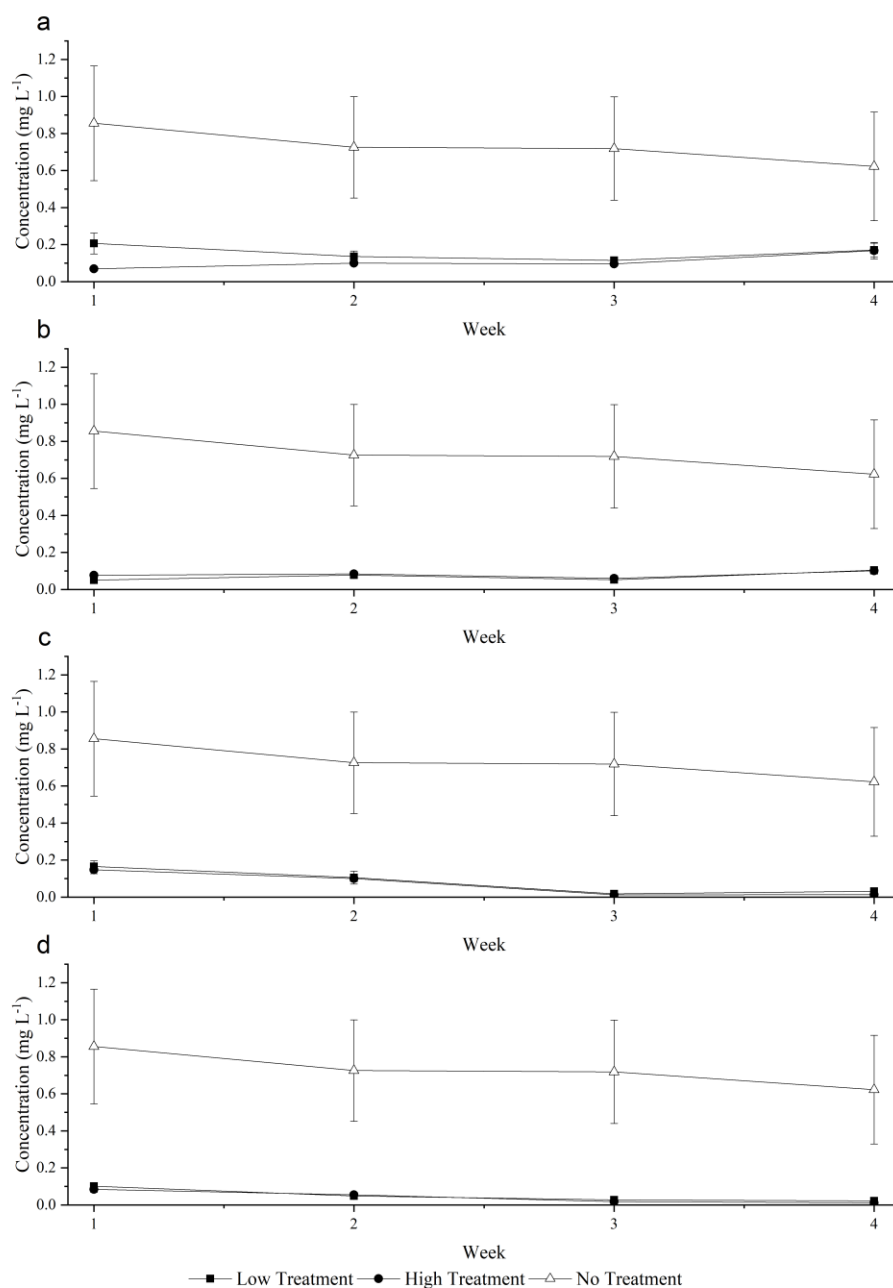


Figure 6-14. Leachate concentrations of Cd in CM (a), SM (b), GWC (c), SCG (d) in the Tyndrum group. n = 4.

The leaching of Cd from the Le Bleymard tailings was significantly increased upon the addition of high application rates of CM and SM and both application rates of SCG (Figure 6-15a-d). The greatest increase in Cd leaching was observed following the high SCG treatment (Figure 6-15 d), which resulted in

an increase of $128 \mu\text{g L}^{-1}$ over the experimental period relative to the un-amended tailing material.

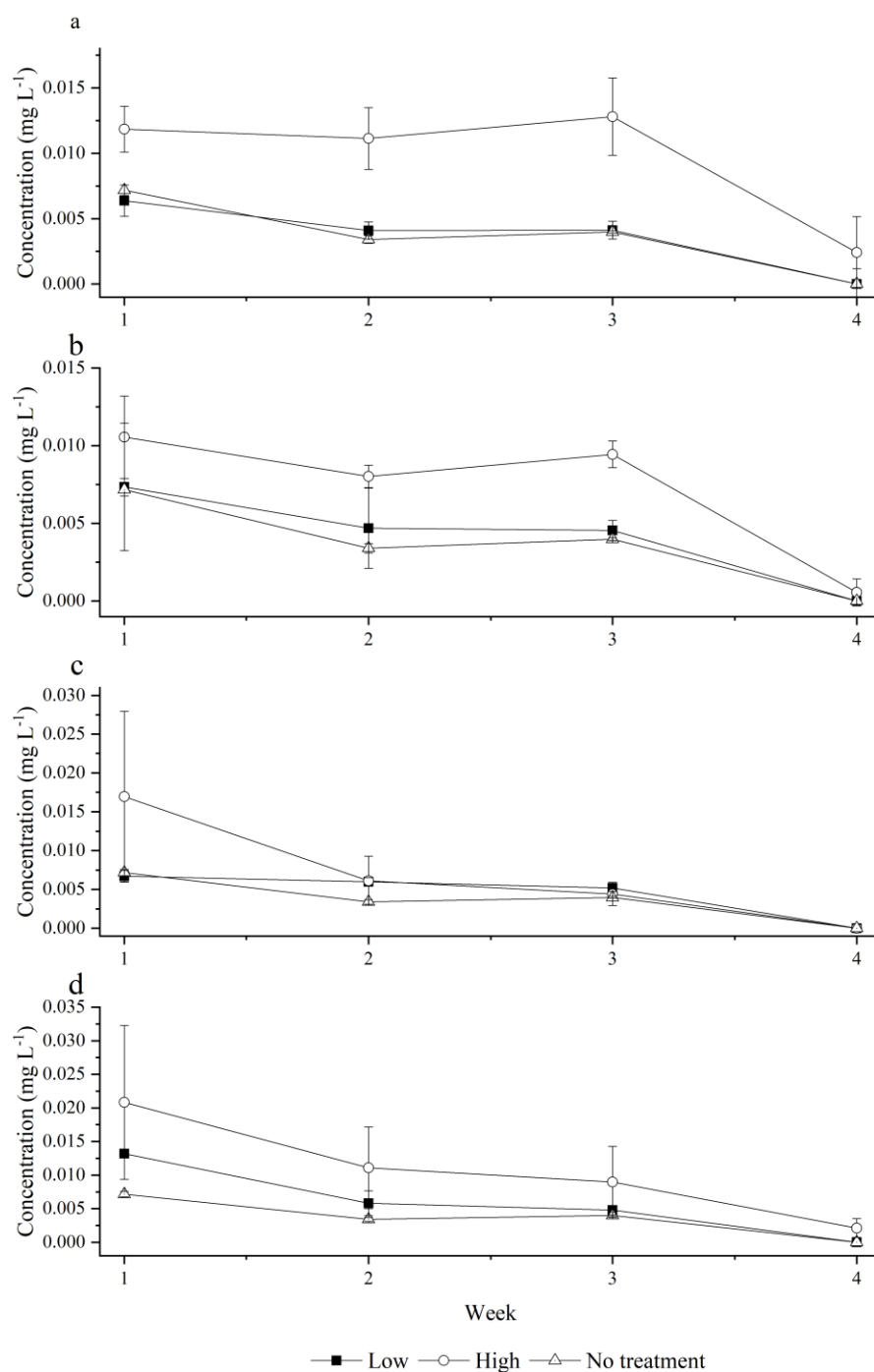


Figure 6-15. Leachate concentrations of Cd in CM (a), SM (b), GWC (c), SCG (d) in the Le Bleymard group. n = 4.

The high application of CM significantly elevated Cd in the leachate of tailing material (Figure 6-15a) ($p = <0.001$). A consistent concentration of $12 \mu\text{g L}^{-1}$ (± 1) was observed until week 4, where Cd in the leachate decreased to $2 \mu\text{g L}^{-1}$ which was not significantly different from the low application rate of CM or un-amended treatment ($p = 0.52$). Similarly, high applications of SM significantly increased the Cd concentration in the leachate in weeks 1 and three by approximately $8 \mu\text{g L}^{-1}$ as compared to the un-amended tailing material (Figure 6-15b), ($p = 0.005$).

Comparing both sites, Cd concentrations in the Le Bleymard tailings were orders of magnitude lower than those found in the Tyndrum samples ($p = <0.001$), with the contrasting findings of the promotion of Cd leaching in the Le Bleymard amended tailings and the reduction in tailings sourced from Tyndrum.

6.6.2 Zn Leaching: Tyndrum and Le Bleymard

The leaching profiles for Zn for Tyndrum and Le Bleymard amended tailings (Figure 6-16 and Figure 6-17 respectively) followed a similar pattern to those of Cd (Figure 6-14 and Figure 6-15). In the Tyndrum tailings, leachate Zn concentrations were significantly lowered following the incorporation of all amendment types across both application rates (Figure 6-16a-d). Concentrations decreased by $\sim 100 \text{ mg L}^{-1}$ at each leaching period ($p = <0.05$). In general applications rates did not affect the removal of Zn from the solid phase material by the leaching solution.

High applications of SM and CM were observed to significantly increase Zn concentrations in the leachate in week four as compared to the previous weeks ($p = <0.05$). There was an increase of $38 (\pm 5.8)$ and $26 (\pm 5.5) \text{ mg L}^{-1}$ for SM and CM respectively (Figure 6-16a-b). Amendment with SCG led to a significantly higher leachate Zn concentration in week one as compared with other amendments (Figure 6-16d) ($p = <0.05$). However, Zn concentrations decreased significantly with time, with concentrations of Zn in both applications less than 10 mg L^{-1} by week 3. Green waste compost treatments had resulted in consistently low concentrations of Zn in the leachate over time (Figure 6-16c).

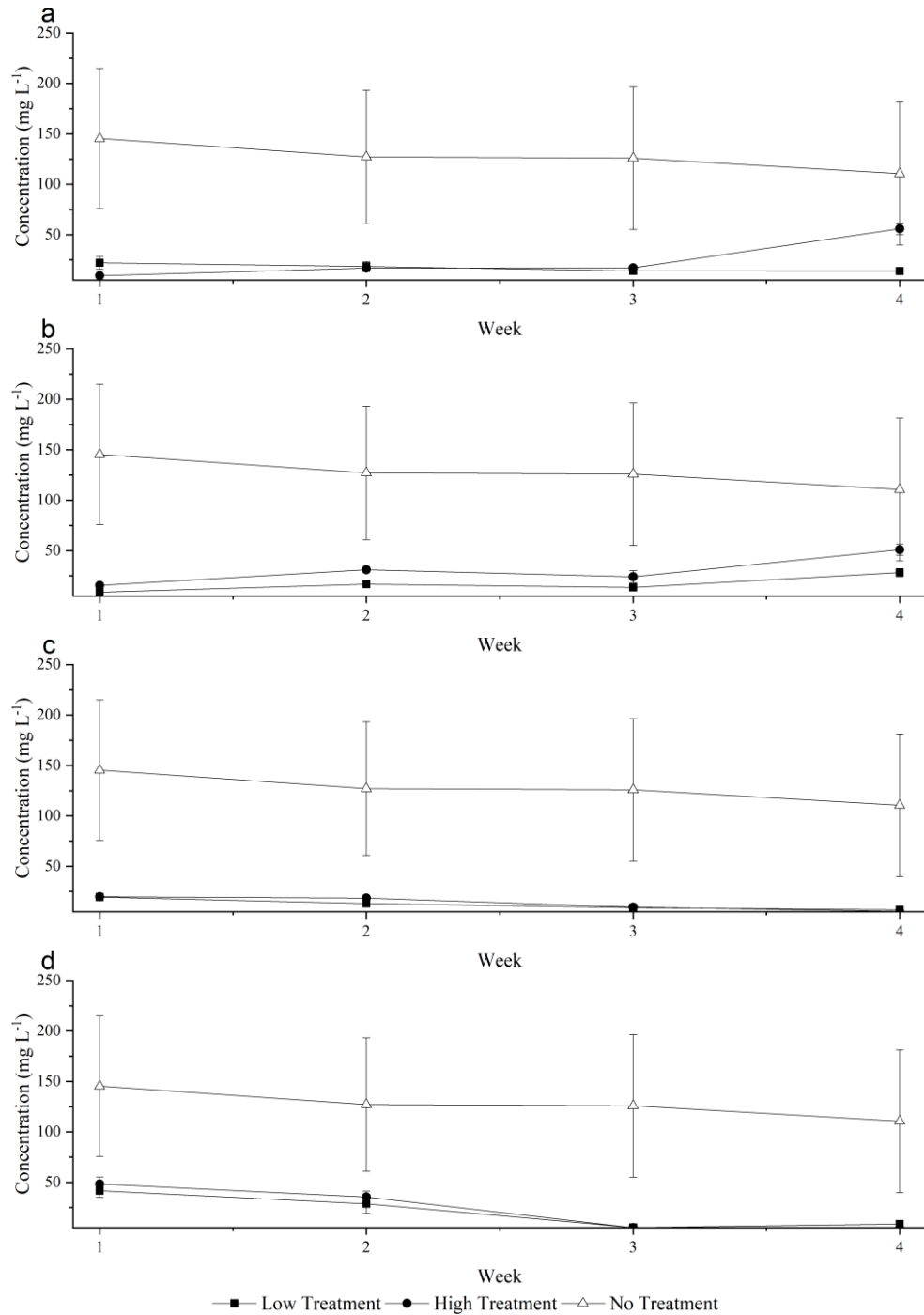


Figure 6-16. Leachate concentrations of Zn in CM (a), SM (b), GWC (c), SCG (d) in the Tyndrum group. n = 4.

In the Le Bleymard-amended tailings high applications of CM led to increases of Zn in the leachate by approximately $1.8 (\pm 0.5)$ and $1.5 (\pm 0.4)$ mg L⁻¹ as compared with the un-amended control and low application rates of CM

respectively ($p = <0.001$) (Figure 6-17a). Concentrations of Zn in leachate collected from SM amended material in both high and low application rates were highly variable in week one, and not significantly different to the un-amended control ($p = 0.151$) (Figure 6-17b). From this point on high applications of SM were $1.00 (\pm 0.13) \text{ mg L}^{-1}$ greater in Zn leachate concentration than the un-amended control ($p = <0.001$) (Figure 6-17b).

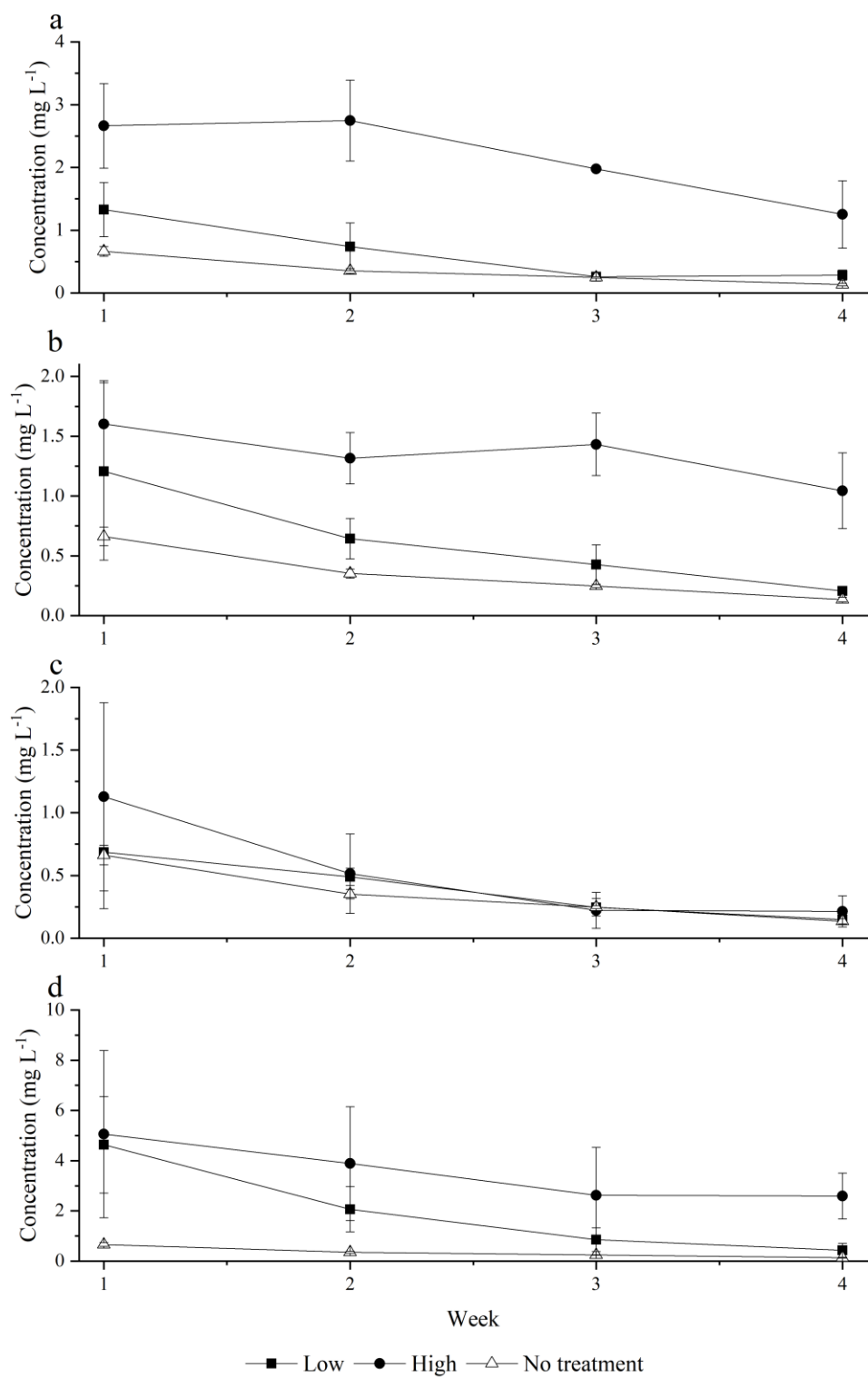


Figure 6-17. Leachate concentrations of Zn in CM (a), SM (b), GWC (c), SCG (d) in the Le Bleyard group. n = 4.

Leachate concentrations of Zn following the high and low applications of GWC were not found to be significantly different from the un-amended tailings ($p = 0.42$) (Figure 6-17c). Zn concentrations in the leachate of tailings amended with SCG were found to be $3.2 (\pm 0.96) \text{ mg L}^{-1}$ greater after the high application rate as compared with the un-amended control throughout the experimental period ($p = <0.001$), with the exception of week 3 ($p = 0.08$). Low application rates of GWC elicited a greater concentration of Zn in weeks one and two ($p = <0.001$), but not in weeks three and four ($p = 0.11$) as compared with the un-amended control (Figure 6-17d). Overall, Zn concentrations in the leachates from un-amended Tyndrum mine tailing material were orders of magnitude higher from the un-amended Le Bleymard material. Following organic amendment incorporation, however, leachate Zn concentrations were significantly lowered for the Tyndrum tailing material, but slightly elevated for the Le Bleymard tailing material.

6.6.3 Pb Leaching: Tyndrum and Le Bleymard

Concentrations of Pb in the leachate from the Tyndrum tailings were significantly increased upon the addition of all organic amendments across both application rates as compared to the un-amended control ($p = <0.05$), with the exception of the GWC treatments (Figure 6-18a-d). Lead leachate concentrations from CM and SM amended tailings increased with time, with higher application rates promoting greater leaching of Pb ($p = <0.05$). In general SM promoted the highest concentration of Pb in the leachate at each time period with the exception of week 4.

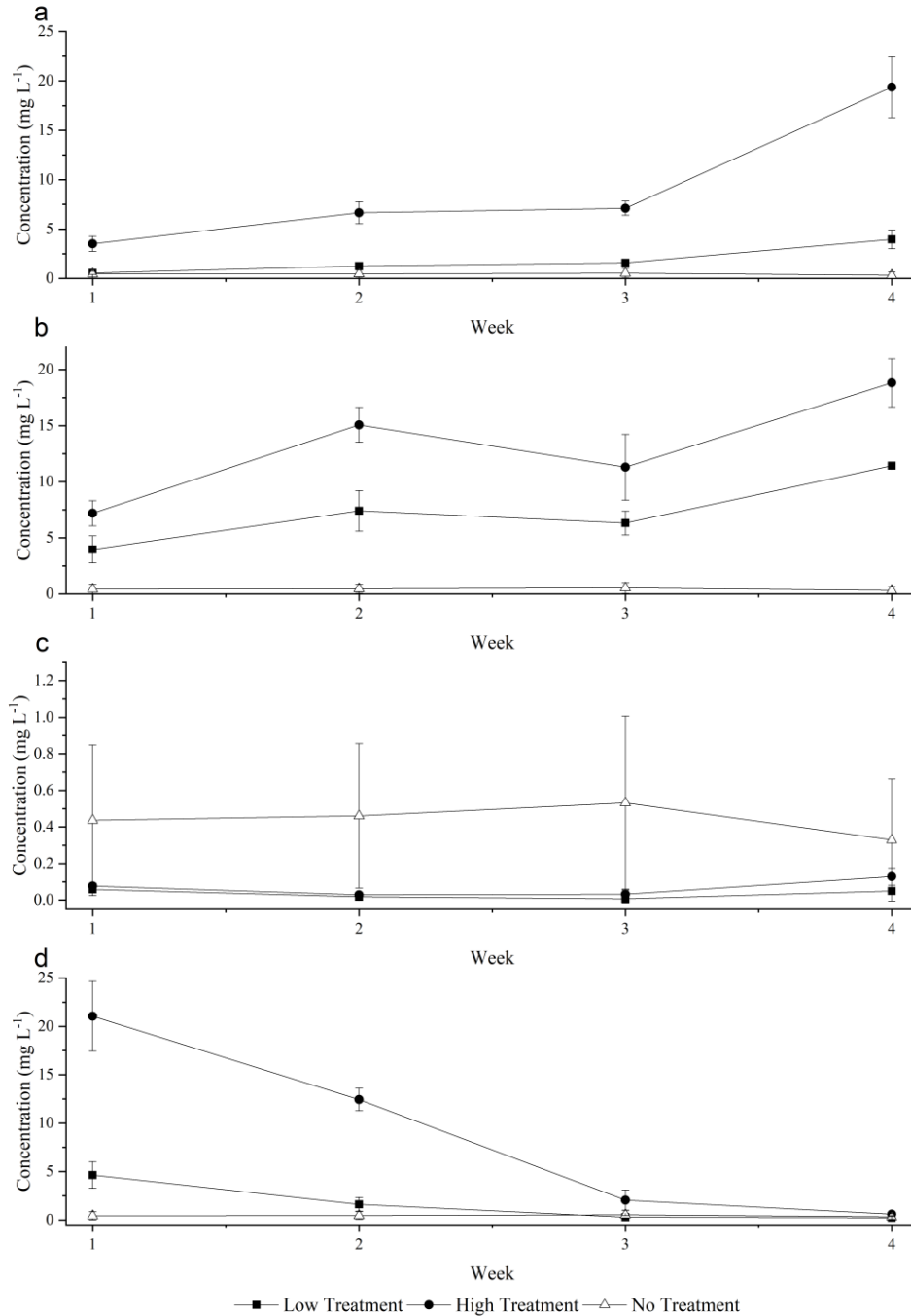


Figure 6-18. Leachate concentrations of Pb in CM (a), SM (b), GWC (c), SCG (d) in the Tyndrum group. n = 4.

Lead leachate from tailing material treated with GWC were significantly lower than the un-amended control over time (Figure 6-18c), demonstrating the ability to decrease Pb leaching from the tailing matrix ($p = <0.05$). No significant differences were observed in the decrease of leachate Pb by high or low GWC

application rates over time ($p = >0.05$). Following amendment with SCG there was a high initial concentration of Pb in the leachate (Figure 6-18d) which declined rapidly over time and was not significantly different from that in the leachate from the un-amended control by week four. Higher applications of SCG were observed to increase the Pb concentrations analysed in the leachate of this material.

In the Le Bleymard group, high applications of SM (Figure 6-19b) and SCG (Figure 6-19d) were found to elicit significantly higher Pb leachate concentrations than the un-amended and GWC groups ($p = <0.05$), with these treatments demonstrating a consistently higher concentration of Pb in the leachate over time. Tailings amended with CM and low application rates of the other groups were not generally found to be significantly different from one another over time, or as compared to the un-amended tailing material ($p = >0.05$).

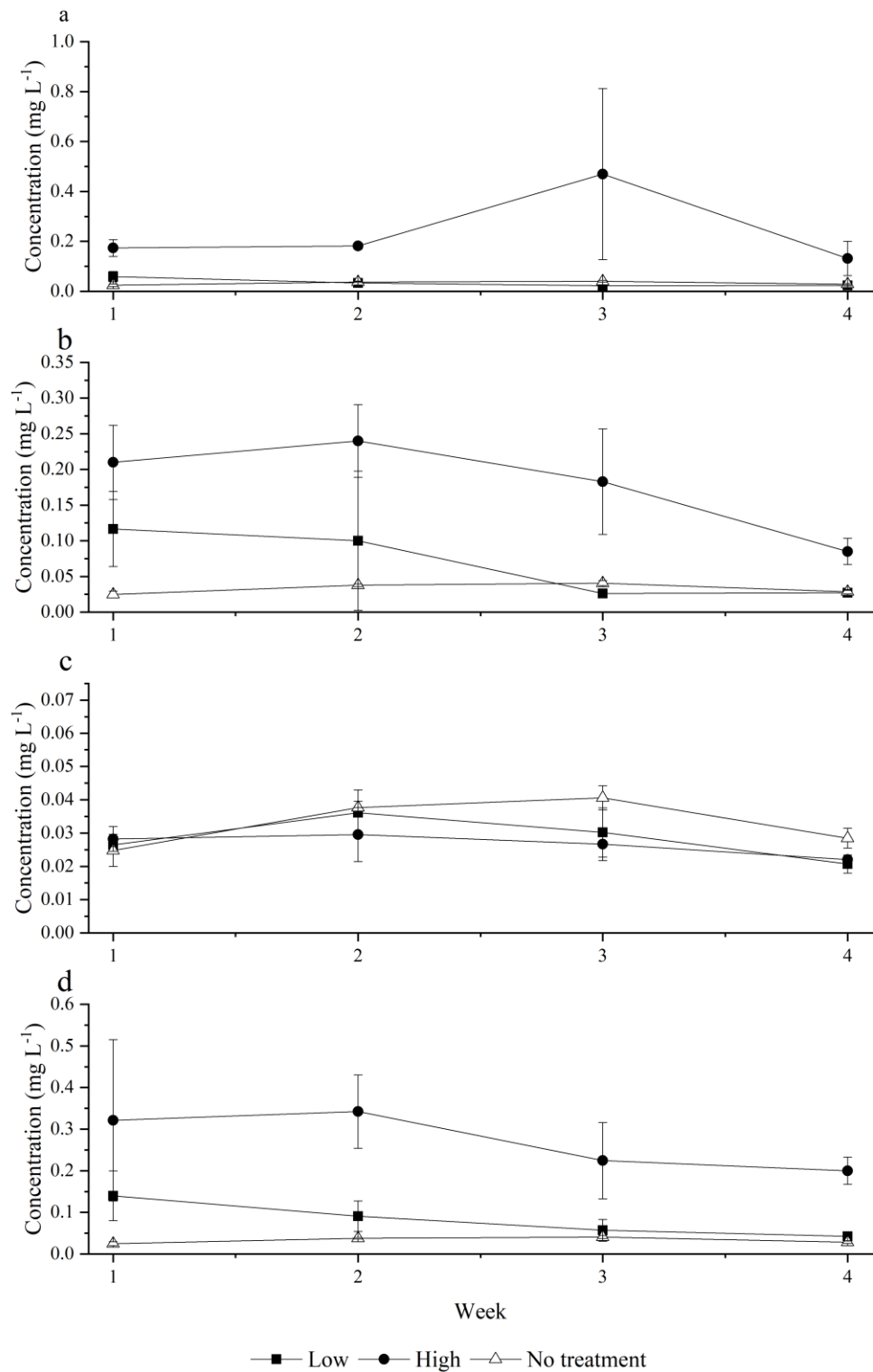


Figure 6-19. Leachate concentrations of Pb in CM (a), SM (b), GWC (c), SCG (d) in the Le Bleybard group. n = 4.

Tailings amended with high and low treatments of GWC were not found to be significantly different from each other or the un-amended group (Figure 6-19c), with the exception of week four, where both GWC treatments were found to produce a decrease in approximately $8 \mu\text{g L}^{-1}$ as compared to the un-amended tailings ($p = 0.02$).

Comparing both tailing sources it is clear that the incorporation of CM, SM and SCG promote leaching of Pb from the tailing matrices, with a significantly higher release observed in the Tyndrum group ($p = <0.05$). However leaching trends are diverse amongst amendment types and tailing sources, indicating that the liberation of Pb is controlled by both tailing and amendment characteristics. Green waste compost treatments were not observed to increase the leaching of Pb in either site and in both cases limited Pb release from the tailing materials investigated.

6.6.4 Principal Component Analysis of Collected Leachate

6.6.4.1 Tyndrum

Principal component analysis of the measured leachate variables in the Tyndrum group are presented in Figure 6-20. Eigenvalues for each analytical output are presented in Appendix 4. PCA plots of individual treatment groups applied to each mine tailings are presented in Appendix 4 alongside their related eigenvalues, which will be discussed in the main body of text.

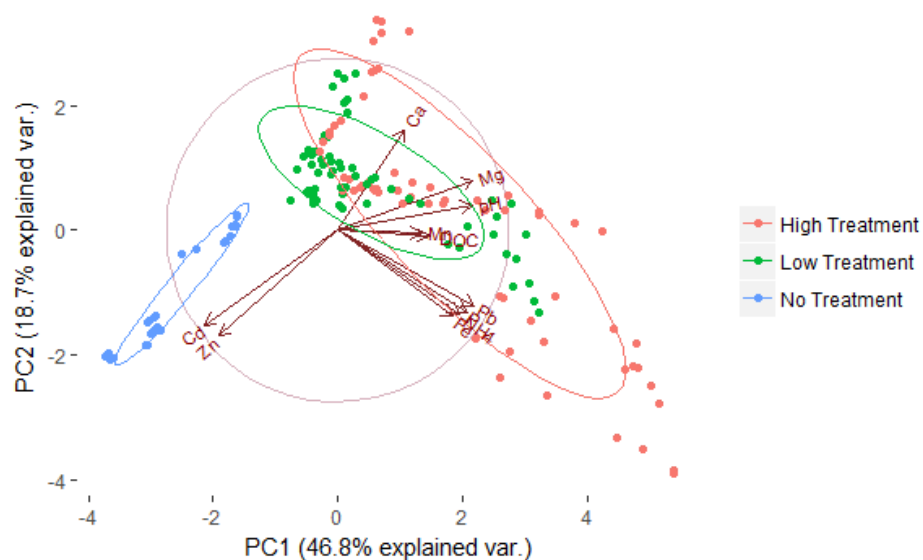


Figure 6-20. Principal component analysis of measured variables in the collected leachate from all treatments in the Tyndrum group.

For the Tyndrum mine tailing material the un-amended control leachates were mostly characterised by higher concentrations of Cd and Zn and lower values of other measured variables. In particular there was a strong inverse relationship between the leachate Ca, Mg and pH and the leachate Cd and Zn concentrations. Grouping of leachate variables suggests a relationship of DOC with Mn and associations with P, Pb, Fe and $\text{NH}_4^+\text{-N}$. Applications of organic amendments in general were found to increase the concentrations of other measured leachate variables, with the clustering of high and low applications (Figure 6-20) demonstrating the increase in Ca, pH and Mg and, in the case of the higher applications, a greater increase in P, Pb, Fe and $\text{NH}_4^+\text{-N}$.

Eigenvalues for the first four components of the treatments applied to the Tyndrum tailings (accounting for 89.4% of the total variance) were considered to represent (i) a decrease in Cd and Zn concentrations relative to measured variables, (ii) an increase in concentration of Ca and the inverse finding for Cd and Zn, (iii) the related increase of DOC and Mn and (iv) the inverse relationship between Ca, Mg and pH.

6.6.4.2 Le Bleymard

Principal component analysis of the measured leachate variables in the Le Bleymard tailing material are presented in Figure 6-21. Eigenvalues for each analytical output are presented in Appendix 5.

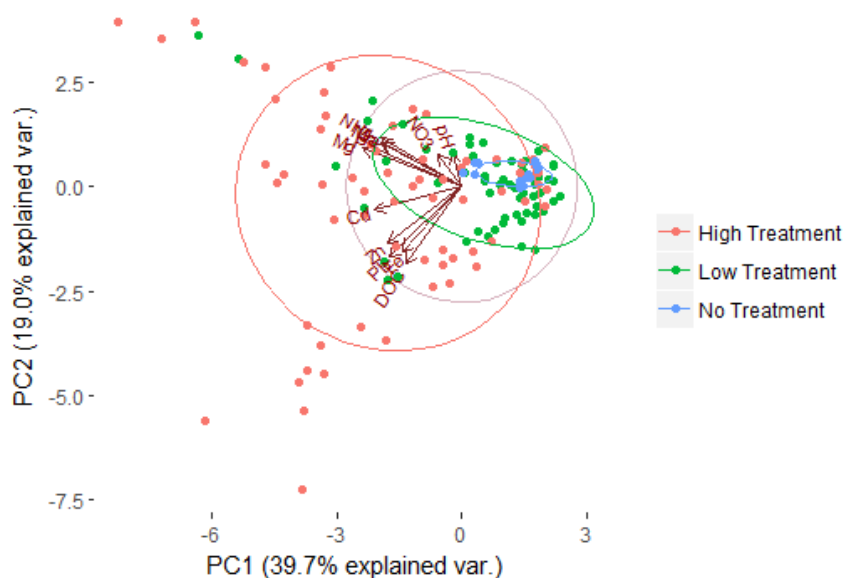


Figure 6-21. Principal component analysis of measured variables in the collected leachate from all treatments in the Le Bleymard group.

The clustering of treatment groups demonstrates a relationship of low concentrations of measured leachate variables with the un-amended control, with the incorporation of organic amendments producing a highly variable effect on the concentrations of measured characteristics, particularly in the high treatment group. Associations between Pb, Fe, Zn and DOC were identified alongside the grouping of Ca, Mg, P, NH_4^+ and Mn. A small correlation was noted between pH and NO_3^- in the tailings.

Eigenvalues of the first five components of the treatments applied to the Le Bleymard tailings (accounting for 85.7% of the total variance) were considered to characterise the contrasting increased concentrations of all measured leachate variables in the amended tailings, with the exception of (i) pH, (ii) the positive relationship of DOC and Pb, and (iii) conflicting inverse and positive relationships of pH and NO_3^- .

6.6.5 Discussion

6.6.5.1 Leaching of PTEs from the Tyndrum Tailings

The addition of organic amendments to the mine tailings from Tyndrum prompted several opposing effects. Across all treatments a substantial removal of Cd and Zn was observed, lowering concentrations of Cd and Zn by up to 95% and 90% in the case of GWC applications. Contrastingly, all amendments except for GWC significantly increased the concentration of Pb in the leachate by several orders of magnitude, with varying leaching trends depending on the amendment in question.

Principal component analysis considering both individual and all treatments together identified a consistent decrease of Cd and Zn concentrations with a concomitant increase of pH, Ca and Mg concentrations. The combination of these variables can be reasonably interpreted as an increase in alkaline conditions, with the concurrent increase in base cations and OH^- in the leachate. It is considered, that as pH increased hydrolysis progressed and the subsequent formation of the neutral species $\text{Cd}(\text{OH})_{2(s)}$ and $\text{Zn}(\text{OH})_{2(s)}$ occurred. These species were possibly then removed from the aqueous phase due to the lower solubility ($K_{sp} = 7.2 \times 10^{-15}$ and 3×10^{-17} for $\text{Cd}(\text{OH})_2$ and $\text{Zn}(\text{OH})_2$ respectively) and subsequent precipitation. (Beesley and Marmiroli 2011; Kogbara *et al.* 2012; Houben *et al.* 2013; Liao *et al.* 2016; Vítková *et al.* 2017; Rodríguez *et al.* 2018). More specifically, the decrease in Cd and Zn leaching from CM-amended mine tailings has been reported by (Schwab *et al.* 2007), who found similar inverse relationships between pH and Cd/Zn. Interestingly, in the application of SCG there was also a strong inverse relationship of Mn, Cd and Zn, suggesting that the increased presence of Mn contributed to a reduction of these PTEs. Comparable findings have been described in (Tack *et al.* 2006; Covelo *et al.* 2007; Hindersmann and Mansfeldt 2014; Suda and Makino 2016), where the scavenging of Cd and Zn have been observed by Mn, potentially promoting the removal of these elements from the aqueous phase into immobile Mn-Cd or Mn-Zn compounds.

Higher Pb concentrations were generally accompanied by higher Fe concentrations in the leachate, suggesting that Fe is a significant transport mechanism of Pb from the Tyndrum mine tailings. Iron has been known to increase the solubility of Pb by the dissolution of Fe (oxy)-hydroxides under reducing conditions upon CM introduction to soil (Miller *et al.* 1985; Alloway 2012) however due to the aerobic nature of the experimental conditions this pathway is unlikely.

A more reasonable explanation would be that the introduction of soluble Fe colloids or hydrolysis products of Fe (III) (E.g. $\text{Fe}(\text{OH})_2$ and $\text{Fe}(\text{OH})_3$) were responsible for the increase in Pb, scavenging weakly associated Pb from the solid phase and a resulting shift into the tailing pore water (Hartley *et al.* 2004). Alternatively, organic inputs possibly induced the mobilisation of Fe (III) (oxy)-hydroxides via organic ligands, microbial processes or the presence of organic acids, promoting their dissolution (Sposito 1993; Mikutta *et al.* 2008; Bosch *et al.* 2010; Vodyanitskii and Shoba 2016). Further evidence for this mobilisation pathway is suggested in the higher concentrations of water-extractable Fe found in the animal manures and the inverse findings in the GWC and SGC applications, which demonstrated low or rapidly declining concentrations of Fe in the leachate and a concurrently diminished concentration of Pb. On the other hand, there is evidence that Pb can also be mobilised by significantly elevated concentrations of soluble OM (Romero *et al.* 2005; Hanč *et al.* 2008; Beesley *et al.* 2014b), as observed in the SCG groups, suggesting that in the absence of Fe and exceptionally high concentrations of soluble OM, Pb can be released from the solid phase and mobilised into the tailing solution.

The leaching or immobilisation of PTE from the amended columns present different trends depending on the amendment and element in question. It appears that the application of manures (CM and SM), which are rich in soluble Fe, promote a consistent elution of Pb from the tailing material. Concentrations of total Fe in CM and SM suggests a large pool of Fe ($1,440$ and $1,580 \text{ mg kg}^{-1}$ in CM and SM respectively (Table 6-1)), indicating that the leaching of Pb could be sustained over a long time and therefore present a significant mobilisation of

Pb from the tailings into solution and the wider environment. This is contrasted by the lower concentrations of Fe found in SCG ($\sim 162 \text{ mg kg}^{-1}$), which suggested that the elution of Pb is initially high but short-lived, a trend that can also be attributed to the mobilisation of OM-induced Pb leaching. It is important to note that although the concentrations of total Fe in GWC were orders of magnitude higher than other organic amendments ($\sim 20,000 \text{ mg kg}^{-1}$) the presence of soluble Fe was lower, and thus the concentration of Pb in the leachate of these treatments was proportionally less. Therefore, it appears that Pb mobility is relatively limited in the un-treated Tyndrum tailings, with a significant risk of increased release mediated by the introduction of materials high in soluble Fe.

There is an important consideration to be made on the longevity of the immobilisation effect on Cd and Zn in the amended tailings. As concentrations of these PTE were inversely related to pH, it would be prudent to assume that the acidification of the tailings from nitrification or microbial degradation of OM may release Cd and Zn into the tailing solution at a later time. The pH in the SM and CM treatments suggest a slight reduction at week four, with a proportional increase in Cd and Zn, indicating that the immobilisation effects may not be permanent and are probably facilitated by reversible adsorption reactions onto the solid phase. In contrast, this effect was not observed for GWC or SCG, suggesting that these amendments may be more suited to the immobilisation of Cd and Zn over longer periods of time.

From the investigation several useful conclusions can be drawn. The mechanisms behind the mobilisation of key PTEs have been described for the tailings at Tyndrum, with the mobility of Cd and Zn facilitated by pH and Pb by the introduction of soluble Fe. These findings build upon the work in Chapter 4, suggesting that Pb release from the mine tailings can be facilitated by either decrease in pH and the subsequent dissolution of Pb-carbonates or by scavenging and complexation of soluble Fe-compounds and elevated concentrations of soluble OM. It would appear that the immobilisation of Cd and Zn is a reversible adsorption reaction and therefore may require a sustainable elevation of pH to maintain a reduction of these PTEs in the tailing solution. Conversely, pH-

neutral amendments low in soluble Fe and DOC may be best suited to limit the mobilisation of Pb from the tailings.

6.6.5.2 Leaching of PTE from the Le Bleymard Tailings

The incorporation of organic amendments into the calcareous tailings sourced from Le Bleymard generally increased concentrations of Pb, Cd and Zn in the tailing column leachates, although the concentration increases were arguably environmentally negligible in some cases.

From the multivariate analysis and clustering of treatments (Figure 6-21) it appears that the low application rates of organic amendments do not have a major effect on most measured variables, whereas the higher application rates generally elicited a discernible change in leachate properties. From the PCA on the individual and complete treatment groups it could be considered that the clustering of [Ca, Zn, Cd, and Mg] represents the underlying smithsonite minerals that dominate the tailings (Chapter 4), with the high application of organic amendments inducing a limited dissolution of this mineral into the tailing solution. This would explain the small increases in these elements in the high treatments of both manures and the SCG. Conversely, Pb was consistently attributable to the presence of DOC in the leachate, suggesting that an increased presence of DOC may mobilise and promote the leaching of Pb from the tailing material.

There are several conflicting relationships when attempting to understand the underlying mechanisms of PTE release. In the tailing material treated with CM the association of base cations Cd and Zn is evident, as is the relationship of DOC and Pb, which likely represents the mobilisation of Pb via soluble organics. Furthermore, in this group an inverse relationship between pH and the presence of NO_3^- -N and NH_4^+ -N was found, potentially indicating a change in pH that is driven from the acidifying process of nitrification explained in Section 6.5.1.

However, PCA outputs from SM do not suggest obvious controlling mechanisms for the leaching of PTEs, other than the increase in application of this OM influences the leaching of all measured outputs as compared to the un-amended tailing material. Furthermore, the incorporation of SCG presented an inverse

relationship between Fe and pH, probably indicating the precipitation of Fe (oxy)-hydroxides (Schwertmann 1991; Alloway 2012), as well as a positive relationship with Pb and DOC, suggesting that different organic amendments and application rates can have subtle and contrasting effects on leachate properties. Clustering and PCA analysis in the GWC treatments were not observed to have identifiable mechanisms for differences in leaching properties, although the recurring observation of DOC and Pb was attributable to approximately 10% of the total variance, again giving evidence of Pb mobilisation as a function of DOC. This provides further evidence that GWC acts less dynamically than other organic amendments in terms of influencing the properties of the tailing leachate.

Overall, the increases in PTE concentrations were minimal upon the incorporation of organic amendments, with increases of around $130 \mu\text{g L}^{-1}$ of Cd and 0.25 mg L^{-1} of Pb for high application rates of SCG for example. As previously mentioned, the increase in PTE could be attributed to the increase in DOC and a slight dissolution of carbonate held PTE as a result of the introduction of organic amendments. These increases would not be environmentally significant based on the CLEA (2002) Soil Guideline Values for residential areas and assuming a high solubility of 5% of total concentrations of Pb and Cd.

Unlike the tailings from Tyndrum, adding OM to the Le Bleymard tailing material had a substantially smaller effect on the measured variables in the column leachates. A large controlling factor in this may be the substantial concentrations of base cations and elevated pH, promoting the immediate hydrolysis and precipitation of positively charged ions (Pb^{2+} , Zn^{2+} , Cd^{2+}) to solid surfaces and preventing an increase in PTE mobility.

The relationship of DOC and Pb would suggest that Pb is liberated from the tailing matrix by soluble organic complexes as described in section (6.6.5.1) and explains the higher association of Pb with the SCG which contained significant concentrations of water-soluble C (Table 6-1). Despite high concentrations of soluble C in the SCG, the leachate concentrations of DOC were orders of

magnitude lower, indicating an immobilisation of C in the tailing matrix. This is contrary to expected results as C solubilisation at high pH is well known (Evans *et al.* 2012), however the expected increase in DOC leaching was likely suppressed by the substantial concentration of free Ca^{2+} and Mg^{2+} , resulting in the subsequent removal of soluble organic C from the tailing solution due to Ca or Mg adsorption and immobilisation (Römkens *et al.* 1996; Kerr and Eimers 2012; Sapek 2013).

Considering the long-term leaching risks of PTE from the tailings it is unlikely that the incorporation of organic amendments would promote significant release of Cd, Pb and Zn due to the elevated pH and presence of Ca/Mg, inducing a strong immobilisation effect to PTEs and other tailing properties that may facilitate the movement of these elements. Concurrently, the alkaline conditions may create issues with nutrient availability, with a reduction in Fe, Mn and Zn and elevated Ca and Mg, leading to nutrient deficits and saline stress respectively (White and Broadley 2003; Tabatabai *et al.* 2005; Hänsch and Mendel 2009; Merchant 2010).

6.6.6 Comparison of Tyndrum and Le Bleymard Leaching Characteristics and Conclusions

The incorporation of the same set of organic amendments into tailings sourced from two contrasting mining tailing materials, one from Tyndrum and one from, Le Bleymard has produced polarised findings in terms of the potential for PTE mobilisation. The addition of OM into the Tyndrum tailing material promoted significant changes in the mobility of Pb and Cd/Zn, whereas in the Le Bleymard samples a small effect was observed, even with additions of high OM.

These differences are perhaps mostly attributable to the slightly acidic conditions of Tyndrum and the alkaline, base cation rich characteristics of Le Bleymard. The Tyndrum tailing material will have a reduced capacity of ion adsorption, leading to more chemical interactions in the tailing leachate and therefore a greater mobilisation of PTE and other cations. In contrast, the elevated pH and significant concentrations of Ca^{2+} and Mg^{2+} ions in the Le Bleymard tailings likely inhibited the mobility of PTE and other cations by hydrolysis and

precipitation reactions, directly reducing the solubility of PTE and transport mechanisms such as Fe in the tailing solution. Furthermore, elevated Ca^{2+} and Mg^{2+} in the tailing solution reduced the concentrations of DOC in the leachate, removing other potential pathways for Pb and reducing leaching risks.

Comparing the effects of organic amendments across both sites, CM and SM can increase the leaching of Pb via the introduction of Fe, and to a lesser extent via DOC, whilst limiting Cd and Zn mobility by increasing the pH and promoting hydrolysis and precipitation reactions. Green waste compost had a smaller effect on leaching characteristics, most likely due to the lower leaching of key mobilisation agents such as Fe and DOC. Finally, SCG have been highlighted as promoting the leaching of Pb via the complexation and solubilisation of Pb with organic matter, with a concurrent finding of reducing Cd and Zn via increases in pH and the possible immobilisation of Cd with Mn.

Overall the leaching characteristics and findings align with a similar investigation by (Pardo, Bernal, *et al.* 2014; Pardo, Clemente, *et al.* 2014). The authors found that the application of organic amendment induced the leaching of NO_3^- -N from GWC in a similar manner to the findings of this experiment, and that nitrification did not appear to occur in more acidic tailings. Furthermore, microbial functioning appeared to have improved in the study upon amendment incorporation, with Pardo *et al.*, (2014) reporting similar findings.

Contrasting findings with this paper were observed in the leaching of NH_4^+ -N and the immobilisation of Cd and Zn where in this investigation was inversely related to pH. Pardo, Bernal and Clemente, (2014) found that Cd and Zn leaching did not occur throughout the column whereas this investigation demonstrated a migration of PTE through the length of the tailing matrix.

The main findings and contributions of this section to the wider scientific literature are as follows:

1. Iron induced mobilisation appears to be the dominant mechanism for the leaching of carbonate associated Pb.

2. Significant introductions of soluble organic complexes can mobilise Pb in the absence of Fe.
3. Changes in pH are the underlying mechanisms for the mobility of Cd and Zn, mainly via hydrolysis and precipitation reactions.
4. The impact of adding OM to mine tailings is highly influenced by the underlying concentrations of Ca^{2+} and Mg^{2+} ions.
5. Organic amendments have contrasting effects on PTEs and other measured parameters depending on the underlying tailing characteristics.

6.7 Solid Phase PTE Fractionation and Availability

6.7.1 Low Molecular Weight Organic Acid Extractions of Cd

Concentrations of LMWOA-extractable Cd in the Tyndrum tailings amended with OM varied significantly depending on application rate and amendment type (Figure 6-22. a). Applications of CM significantly increased LMWOA-Cd with the remainder of the treatments statistically similar to the un-amended control. High applications of SM and SCG were found to be similar to the un-amended tailings, with no applications of organic amendment reducing the extractable concentrations of Cd from the solid phase as compared to the un-altered tailing material. Both applications of CM increased extractable Cd by approximately 1.2 mg kg^{-1} in comparison to un-amended columns.

In contrast, the application of organic amendments in the tailings sourced from Le Bleymard significantly reduced the LMWOA-extractable Cd in the solid phase (Figure 6-22. b). The greatest reduction was observed for high applications of SM and GWC, which were significantly lower than both CM treatments and the low application of SM. Concentrations of LMWOA-Cd were reduced by approximately 10 mg kg^{-1} in all applications, with the exception of high SM and GWC treatments, where the reduction was approximately 14 and 15 mg kg^{-1} , resulting in an approximate decrease of 30% compared to the un-amended tailings.

Concentrations of LMWOA-Cd in Le Bleymard in all treatment groups were significantly greater than those derived from the Tyndrum mine tailing material ($p = <0.001$), with the un-amended control sourced from Le Bleymard exhibiting

a difference of approximately 47 mg kg⁻¹. Despite the reductions in LMWOA-extractable Cd following the amendment of tailing material from both locations, the concentrations of Cd remain elevated in comparison to soil guideline values as proposed in the CLEA 2002 guidance.

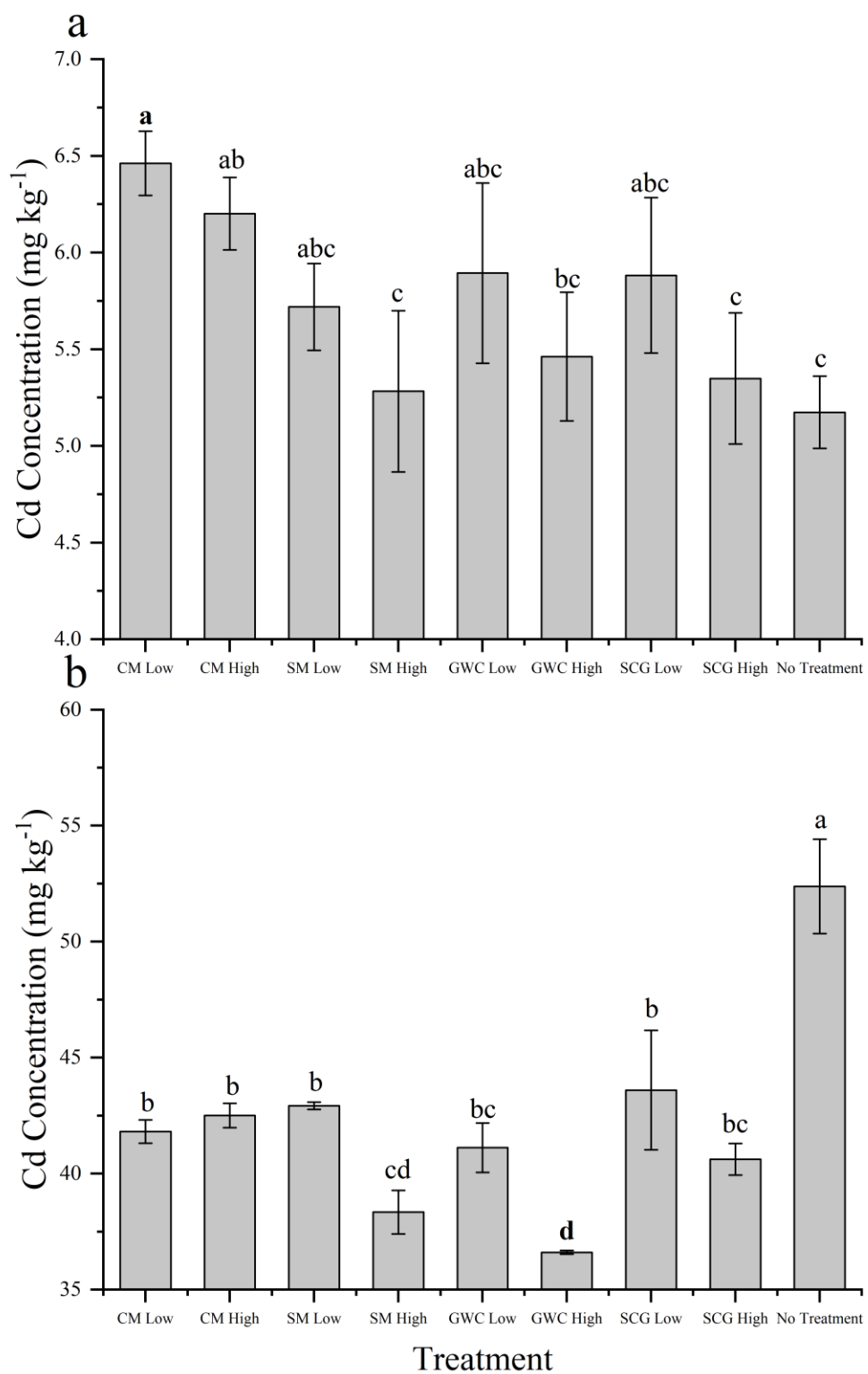


Figure 6-22. LMWOA extractable Cd from a) Tyndrum group and b) Le Bleymard group. Means that do not share a letter are significantly different ($\alpha = 0.05$). CM = cattle manure, SM = sheep manure, GWC = Green waste compost, SCG = spent coffee grounds.

6.7.2 Low Molecular Weight Organic Acid Extractions of Pb

Applications of SM and GWC in the Tyndrum group significantly reduced LMWOA-extractable Pb, with increasing application rates producing a greater decrease of Pb (Figure 6-23. a). High application rates of SM and GWC reduced LMWOA-Pb by approximately 2,297 and 2,092 mg kg⁻¹, which accounted for a 32-36 % decrease respectively. Lower applications had a smaller effect, with a reduction of 1,241 (19.2%) and 1,380 mg kg⁻¹ (21.4%) from the low incorporation of GWC and SM respectively.

Tailing material amended with CM and SCG were not significantly different in LMWOA-Pb as compared to the un-amended control, with no differences observed between either amendment or the rate of application.

The decrease in LMWOA extractable Pb in the Le Bleymard group was significantly reduced in almost all treatment groups, with the only exception observed in low application rates of SM (Figure 6-23. b). The most pronounced reduction of LMWOA-Pb was for the high application rate of SM and GWC, with reductions of approximately 630 (24%) and 801 (31%) mg kg⁻¹. Other amendments reduced LMWOA-Pb by smaller proportions, with the lowest significant reduction observed for the low application of SCG, demonstrating a removal of 333 (12%) mg kg⁻¹ as compared to the un-amended tailings. The application rate of amendments did not produce significant differences in Pb reduction with the exception of GWC and SM, where increasing application rates elicited a greater removal of Pb from this fraction.

Concentrations of LMWOA-Pb were significantly higher in the Tyndrum tailing material across all treatments as compared to the Le Bleymard group ($p < 0.001$). In the un-amended tailings Pb was approximately 3,800 mg kg⁻¹, indicating a higher baseline of Pb in the Tyndrum columns.

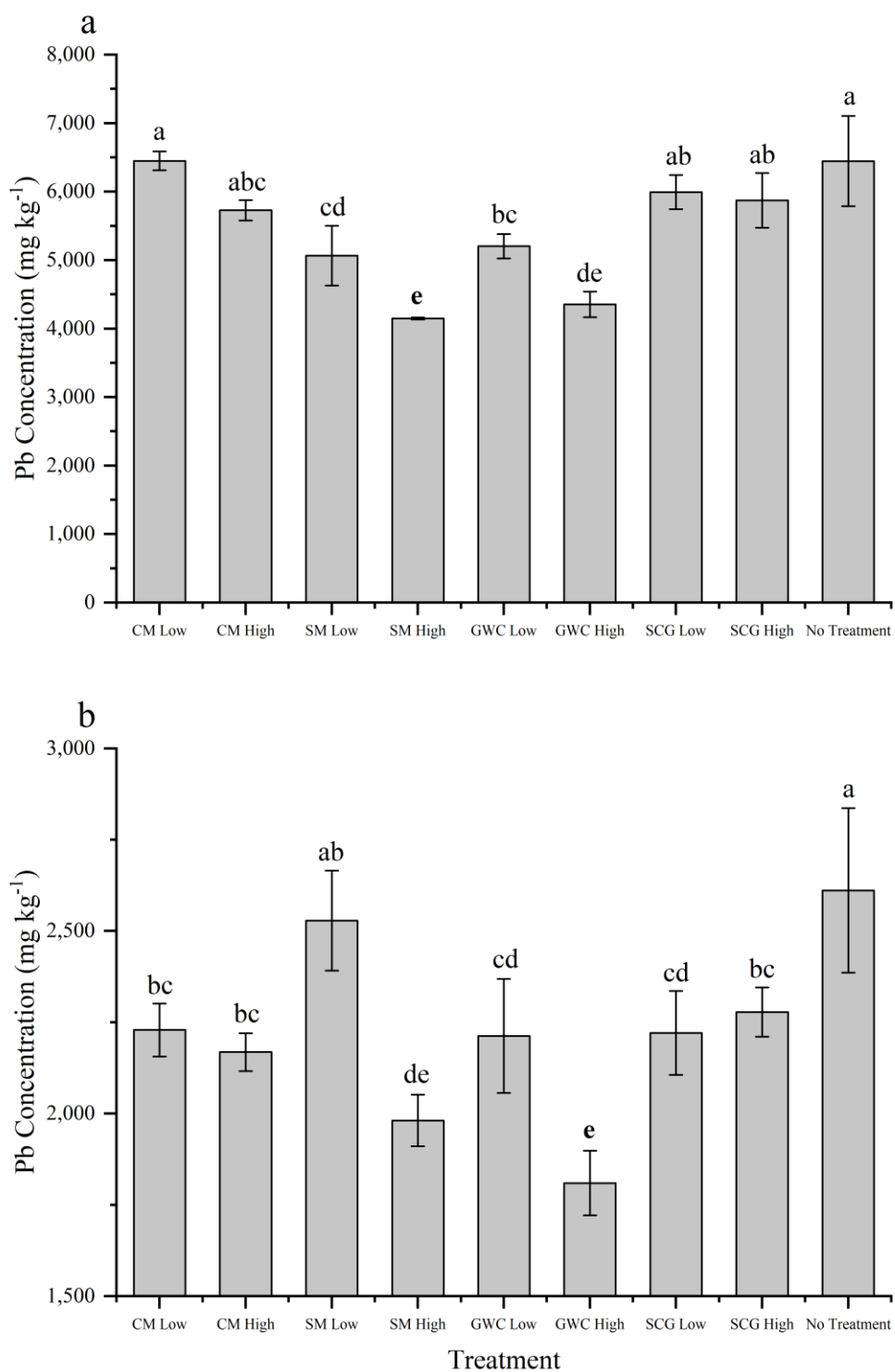


Figure 6-23. LMWOA extractable Pb from a) Tyndrum group and b) Le Bleymard group. Means that do not share a letter are significantly different ($\alpha = 0.05$). CM = cattle manure, SM = sheep manure, GWC = Green waste compost, SCG = spent coffee grounds.

6.7.3 Low Molecular Weight Organic Acid Extractions of Zn

For the Tyndrum tailings, all application rates of all organic amendment treatments significantly decreased LMWOA-Zn with the greatest differences observed between the GWC, SCG and SM treatments and the un-amended control (Figure 6-24. a). The high application of SCG promoted the largest reduction in LMWOA-Zn, (1,622 mg kg⁻¹, 28.7%) followed by low rates of SM (1,451 mg kg⁻¹, 25.7%) and GWC (1,341 mg kg⁻¹, 23.7%). The lowest reduction in LMWOA-Zn was observed for the low incorporation of CM, reducing Zn by 1,200 (12.4%). Overall, significant decreases in LMWOA-Zn were seen for the Tyndrum group.

Significant differences between the tailing types were observed in most treatment groups ($p = <0.05$), with the concentrations of LMWOA-Zn generally higher in the columns from Le Bleymard. No significant differences between both application rates of GWC and the low application rate of SM were observed ($p = >0.05$). Concentrations of LMWOA-Zn were approximately 200 mg kg⁻¹ higher in the un-amended tailings from Le Bleymard as compared to Tyndrum, indicating a fairly similar concentration of Zn.

The incorporation of high applications of SM, GWC and both application rates of SCG significantly lowered LMWOA-extractable Zn in comparison to the un-amended control in the Le Bleymard group (Figure 6-24.b). The addition of CM and low application rates of SM and GWC to the tailing material were not observed to be significantly different from that of the un-amended material.

The reduction in LMWOA-Zn in the Le Bleymard group for SCG treated tailings was 554 (9.5%) and 718 (12.3%) mg kg⁻¹ for low and high applications respectively, with a smaller reduction observed in the high SM (426 mg kg⁻¹, 7.3%) and high GWC applications (395 mg kg⁻¹, 6.8%).

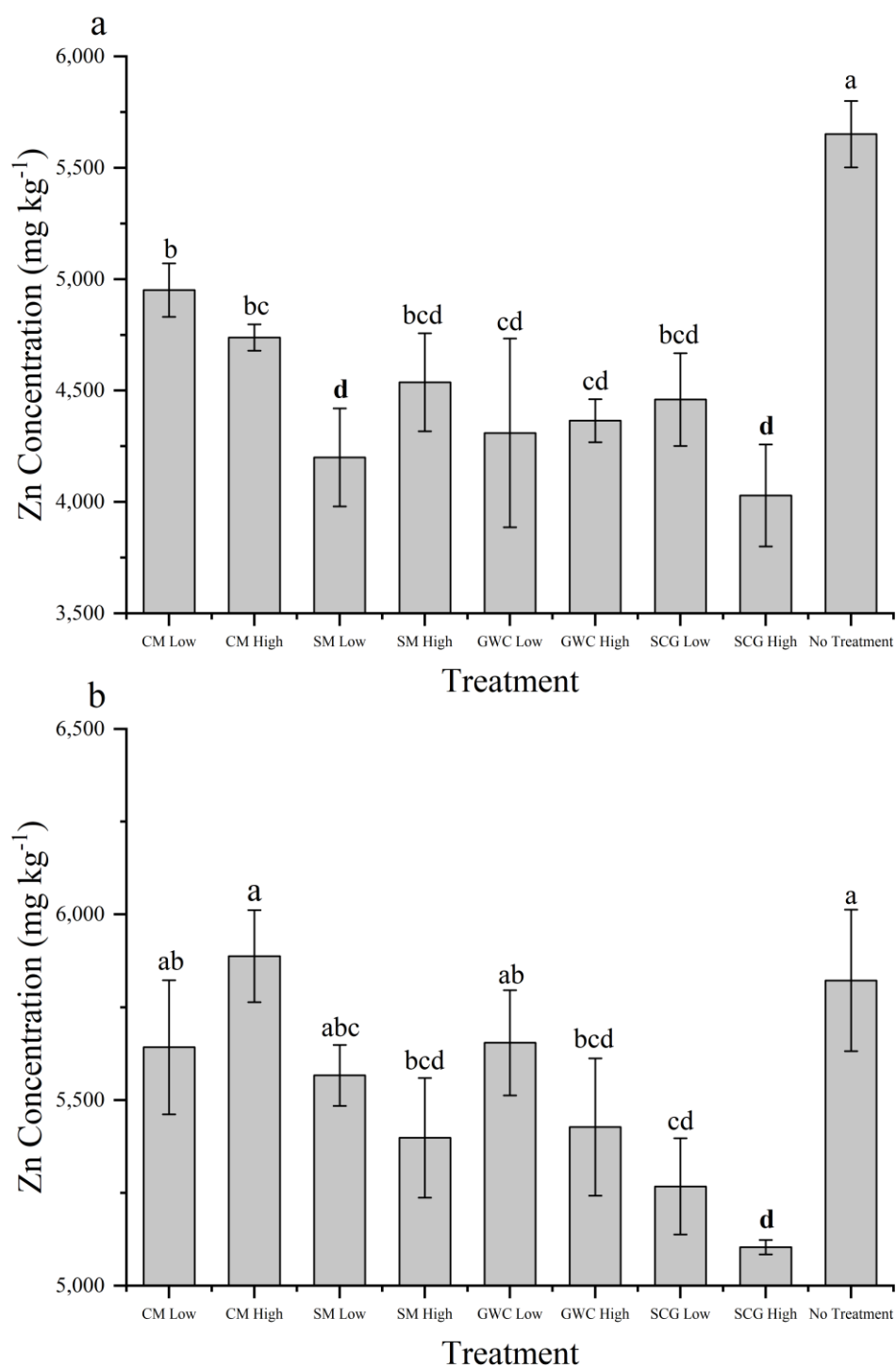


Figure 6-24. LMWOA extractable Zn from a) Tyndrum group and b) Le Bleymard group. Means that do not share a letter are significantly different ($\alpha = 0.05$). CM = cattle manure, SM = sheep manure, GWC = green waste compost, SCG = spent coffee grounds.

6.7.4 BCR-Fractionation of Amended Tyndrum Tailings

Results from the BCR extracted tailings for Cd, Pb and Zn are presented in (Figure 6-25). Outputs from the Analysis Of Means (ANOM) are presented in Appendix 6, with all significance tests performed at $\alpha = 0.05$.

From the ANOM, pools of Cd were generally not altered by the incorporation of organic amendments as compared to the un-amended control (Figure 6-25. a). Cadmium mainly resided in the organic/sulphide and residual fractions of the tailing matrix, with significantly larger proportions of Cd in these fractions in comparison to the weakly associated and reducible pools. Considering interaction effects, the proportions of Cd in the oxidisable pool of the high SCG treatment were significantly higher than the grand mean of other treatments, suggesting a greater proportion of Cd in this fraction. Concurrently, this treatment group had significantly lower proportions of Cd in the residual phase in comparison to other treatments.

Lead was mainly found in the weakly associated fraction (Figure 6-25. b) in the amended and un-amended columns, with the un-amended tailings exhibiting 74% (± 5.0) of Pb in this fraction.

From ANOM, the un-amended tailings had a significant interaction of Pb in the weakly associated fraction, indicating that the application of organic amendments significantly reduced the proportion of Pb in the weakly associated fraction, with up to a 19.6% reduction for the high application of SCG. Furthermore, the application of organic amendments significantly increased the proportion of Pb in the reducible phase in comparison with the un-amended tailings, with the greatest proportion observed for the high application of SCG. The oxidisable and residual fractions were not significantly different between treatment groups and were observed to attribute approximately 13.5 and 10.1 % of the total concentration of Pb in the tailings. No significant differences were observed in the total concentrations of Pb between treatment groups, suggesting a change in chemical fractionation rather than a loss of Pb.

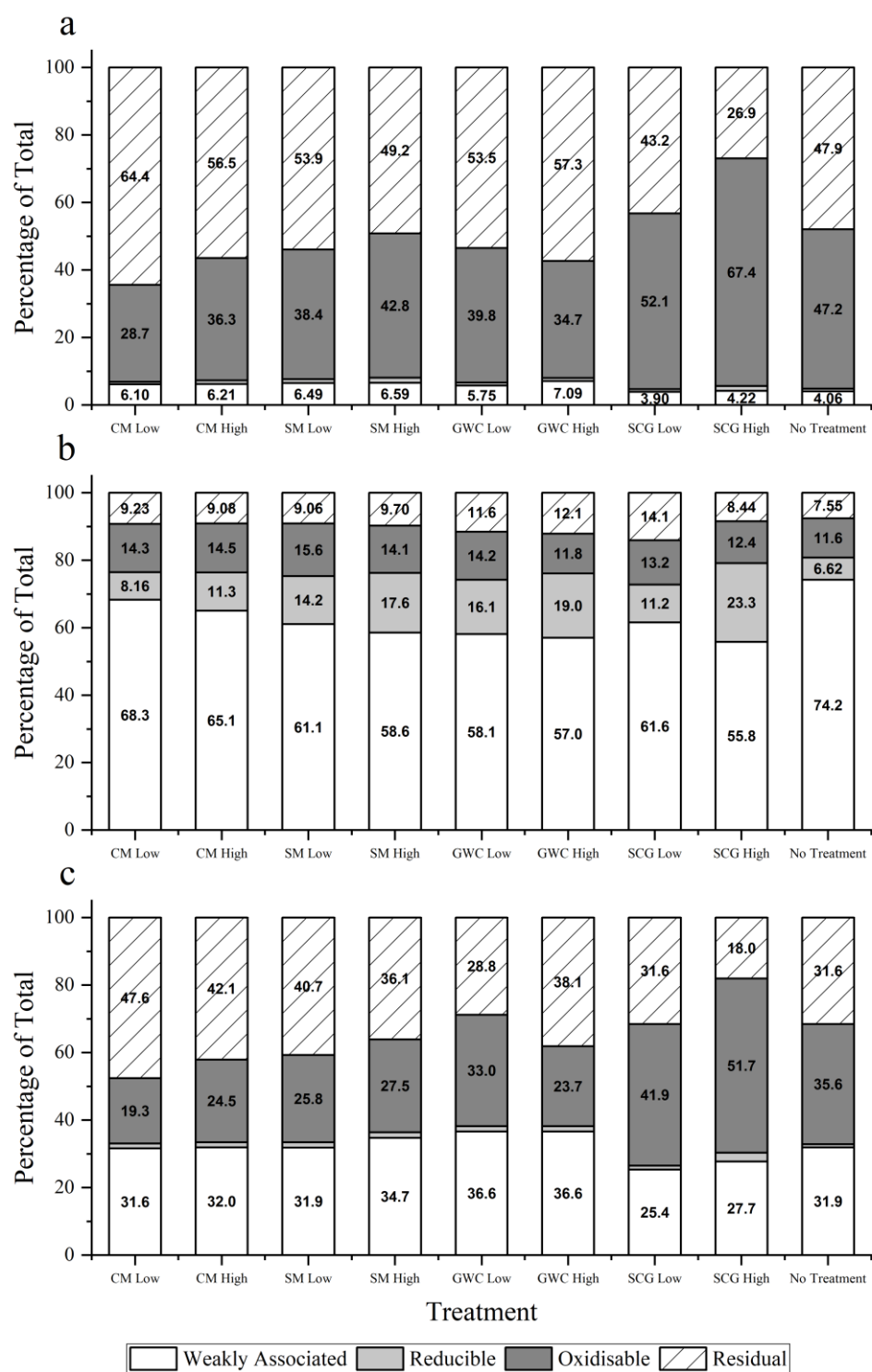


Figure 6-25. Percentage of total concentrations BCR Fractionation of amended Tyndrum tailings. a) Cd, b) Pb and c) Zn. CM = cattle manure, SM = sheep manure, GWC = Green waste compost, SCG = spent coffee grounds.

Zinc was found to reside fairly uniformly between the weakly associated, oxidisable and residual fractions in the un-amended tailings (Figure 6-25. c), with approximately 0.9% attributable to the reducible phase. In terms of differences in treatment group, the largest effect sizes were observed in the SCG and un-amended tailings.

The application of SCG increased the proportion of Zn in the oxidisable phase in both high and low application rates, with the high rate also contributing to a reduction of 6.8% of Zn in the weakly associated phase and an increase of approximately 1% in the reducible pool in comparison to other treatments. The application of all organic amendments significantly increased the concentrations of Zn in the reducible fraction although the effect was small, with a mean increase of $0.7 (\pm 0.2) \%$ across all treatment groups and application rates.

Low applications of CM were found to have significantly lower proportions of Zn in the oxidisable phase and higher proportions in the residual phase in comparison to other treatments, with an approximate difference of 10% for each fraction. A similar finding was observed for the high application of GWC, where a reduction of 9% of Zn in the oxidisable phase was observed in comparison to the other treatment groups and un-amended control.

Significant differences in total Zn concentration were observed between treatment groups, with the control exhibiting the highest total Zn concentrations and SM demonstrating the lowest (Appendix 6).

6.7.5 Discussion

The introduction of organic amendments into the tailing materials had a range of effects on the extractability and fractionation of PTE in the solid phase.

6.7.5.1 Cd

Low molecular weight organic acid extractable Cd was increased by the addition of both low and high application rates of CM and not significantly reduced in any treatment in comparison to the control. Total Cd in the CM amendment treatment was $< 0.003 \text{ mg kg}^{-1}$, and so could not have contributed to the increase

observed in the LMWOA fraction, rather, it is probable other factors promoted the mobility of Cd within this treatment.

In the wider literature, numerous studies have demonstrated the weaker participation of Cd in binding to organics, Fe/Mn/Al (oxy)-hydroxides and soil particles as compared with other PTE such as Pb, Zn and Cu (Alloway and Jackson 1991; Boekhold and Van der Zee 1991; Yuan and Lavkulich 1997; Prokop *et al.* 2003; Bolan *et al.* 2004; Karaca 2004; Qi *et al.* 2018), highlighting the relative mobility of this element in comparison to Pb and Zn in the study and the lack of sorption of Cd to organic ligands. Furthermore, competition from other cations for bonding sites and increased solubility from Cd-Cl formation have also been described (Bingham *et al.* 1984; Smolders *et al.* 1998; Covelo *et al.* 2007; Shaheen *et al.* 2017). It is possible that in the amended Tyndrum tailings a proportion of Cd was held by weak, electrostatic bonds on tailing surfaces and by easily disassociated organic matter, which under the acidic conditions of the LMWOA-extraction was then released into the analytical solution.

This proposition is supported by the BCR-fractionation data which did not demonstrate significant differences in the weakly associated or reducible fractions of Cd in contrast to the findings of Pb and Zn. This finding may have several concurrent explanations. Firstly, the labile nature and tendency of Cd to participate in weakly associated bonding, rather than stronger inner sphere processes, may be an intrinsic factor in the lack of Cd retention by the amended tailings. Secondly, the availability of free binding sites presented by the introduction of organic amendment may have been consumed by the stronger competitive effects of Pb complexation with organics, especially since total Pb concentrations were orders of magnitude higher than Cd. Finally, Cd is not known to undergo ‘soil aging’ whereby mobility decreases over time (Tang *et al.* 2018; Zhang *et al.* 2018), suggesting that Cd sorption reactions are reversible and that a permanent reduction in availability is unlikely in these conditions. On a side note, the inverse relationship between Cd and Mn observed in the leachate of SCG was mirrored in a slight reduction of Cd in the weakly associated state

and an increase in the reducible phase, supporting the previous proposal of Cd co-precipitating with Mn. The potential immobilisation effect however was small and did not contribute significantly to the overall distribution of Cd in the tailing matrix.

Contrastingly, in the Le Bleymard group the application of organic amendments significantly reduced the concentrations of LMWOA-extractable Cd by up to 30%, indicating a strong removal of Cd from the LMWOA-fraction. As the LMWOA extractions is based on the use of weak organic acids, the extraction process likely liberated carbonate bound Cd from the tailing material, this was equivalent to approximately 30% of the total Cd concentration in the tailing material. Despite the weak binding of Cd to organic constituents discussed in the previous paragraphs it is likely that the presence of OM enhanced the formation of Cd to more stable organic complexes, such as those found in the high treatment of GWC, which exhibited the greatest reduction of Cd as compared to other treatments. This may have been partially due to a lower presence of Pb and an increased presence of Cd in the Le Bleymard tailings, limiting competitive pressure for binding spaces and resulting in a larger change in the mobility of this element.

Similar findings of reduced Cd mobility upon the addition of organic amendments, such as GWC, have been reported (He and Singh 1993; Kaschl *et al.* 2002; Shuman *et al.* 2002; McLaughlin *et al.* 2006; Hanc *et al.* 2009; Elbana *et al.* 2018), with the authors suggesting that, despite a lower affinity for organic matter, Cd immobilisation and increased sorption to the solid phase can occur to a certain extent in soils with intrinsically low OM content and high Cd. The decrease of Cd in the amendments applied at Le Bleymard can then be considered more permanent than the findings at Tyndrum as a proportion of the total Cd was not re-mobilised upon interaction with an acidic solution.

It is important to note that despite a reduction in LMWOA-Cd in the Le Bleymard tailings a significant concentration of Cd in this fraction remains, demonstrating the risk of Cd availability if the tailings were to be subjected to acidic conditions, such as those produced by plant roots.

6.7.5.2 Pb

Lead concentrations under LMWOA-extractable conditions were significantly lowered upon the higher incorporation rate of SM and GWC, indicating a greater effect in decreasing labile Pb for these amendments. From these results it can be considered that the introduction of these particular organic materials can immobilise approximately 30% of mobilise Pb if the carbonate-bound tailings were to undergo dissolution, whereas the remaining treatments and application rates do not appear to have a significant immobilisation effect on Pb in the tailings.

The findings from the LMWOA extraction contradict the comparable findings of the first fraction of the BCR procedure. Both procedures are based on the shaking of tailing material in a weak, predominantly acetic acid-based solution for 16 hours at ambient conditions, and so similar outputs would be expected. However, the BCR procedure suggested that SCG did have a significant effect on the decrease of Pb from the weakly associated fraction. The differences in the procedures are likely due to the higher extractive power of the BCR-1 procedure (LMWOA = 0.01 M L⁻¹ acetic acid, BCR-1 = 0.11 M L⁻¹) and thus a greater liberation of Pb was observed. This suggests that SCG have a larger capacity to immobilise Pb than described in the LMWOA extraction, which may underestimate the transformation capacity of SCG in lower conditions of available Pb. Furthermore, the effect of transformation of high applications of GWC, SM and SCG were not significantly different under the BCR-1 procedure.

Considering the BCR fractionation of Pb, upon organic amendment incorporation there was a decrease in the proportion of Pb in the weakly associated fraction as compared to the control and a resulting increase in the reducible fraction, with the greatest differences observed for the high application of SCG. The reducible fraction is characterised as PTE bound to Fe/Mn and so the data suggests that there was an incorporation of labile Pb into this pool and a decrease in relative bioavailability of Pb. There are several possible reasons for this: one could argue, that reducing conditions promoted the solubilisation of Fe/Mn which were able to scavenge liberated Pb²⁺ from the tailing solution, with

the eventual precipitation of Fe/Mn – Pb oxy-hydroxides and the removal of Pb from the weakly associated fraction. However, the aerobic conditions observed throughout the experiment (Section 6.4.3) suggest that this process was not occurring. Alternatively, the dissolution of carbonate held Pb probably interacted with reactive Fe compounds introduced into the tailing solution, with a proportion of this Fe-scavenged Pb lost via leachate and the remainder immobilised by the formation of stable Fe and Mn precipitates.

It is possible that Mn had a greater function in the stabilisation of Pb as compared to Fe as Mn was not associated with the leaching of Pb (section 6.6.4.1) and has been demonstrated to have a greater sorption capacity for Pb than Fe (McKenzie 1980; Dong *et al.* 2000; Wang *et al.* 2012; McCann *et al.* 2015). This would further explain the higher incorporation of Pb into the reducible pool in the SCG treatments as Mn concentrations in the leachate were significantly higher in this treatment group.

The remaining oxidisable and residual fractions were not significantly different in any of the treatments, and unlikely to participate in chemical reactions due to the inherently high stability of these pools of Pb. Overall, the BCR procedure has supported the findings of Pb mainly existing as weathered Pb-carbonates, rather than the original PbS ore that was mined at Tyndrum (Pulford *et al.* 2009).

In the Le Bleymard group LMWOA-extractable Pb was significantly decreased upon the addition of all organic amendments with the exception of low rate applications of SM. It is possible that the immobilisation of Pb in the Le Bleymard tailings followed the same pathway as in Tyndrum, with a sorption of Pb onto Fe/Mn hydroxides or possibly precipitation as more stable compounds. However, the high pH and low concentration of Fe/Mn in the tailing solution may have limited the interaction of Pb with Fe/Mn due to adsorption onto tailing surfaces, therefore this route of immobilisation may not be as likely.

The highest reduction of LMWOA-Pb was found in the high GWC and SM treatments, which for the former could be considered as exhibiting more stable organic compounds and the possibility of the formation of chemically stable

complexes with Pb. Sheep manure may also have sufficient quantities of stable binding sites to promote this effect. Overall, as Pb is known to bind strongly to organic matter via inner sphere formations (Alloway 2012) it is suggested that a proportion of the total Pb in the tailings reacted in this way with the amendments. SCG may not have as high proportions of stable binding sites due to the high concentrations of easily extractable C, therefore exhibiting a limited ability to retain Pb when exposed to acidic conditions.

Overall, it is clear that the incorporation of organic amendments have the capacity to limit the concentrations of weakly bound Pb in calcareous and acid-neutral mine tailings, however it must be noted that a significant concentration of Pb remains in these extractable fractions, indicating the limited capacity of organic amendments to decrease the potential mobility of carbonate associated Pb under acidic conditions.

6.7.5.3 Zn

Concentrations of LMWOA-extractable Zn were lowest for the high applications of SM and SCG in the amended Tyndrum tailings, with a reduction of up to 12% in the latter. The reduction in Zn concentrations were significantly lower in comparison with that for Pb, but higher than that of Cd. Considering the weakly associated fraction of the BCR procedure, the findings were comparable with the LMWOA approach, indicating a reduction of approximately 7% in the SCG treatments. Furthermore, although increases of Zn in the reducible fraction were significantly different, the chemical shift into this pool was generally less than 1% indicating a small increase in Zn into this phase.

Zinc is known to have generally exhibit a stronger affinity for organic ligands and Fe/Mn oxy-hydroxides than Cd, but less so than Pb in soil systems, indicating that Zn will compete for binding sites to a greater extent than Cd but will be displaced if excessive concentrations of Pb are present (Echeverría *et al.* 1998; Vega *et al.* 2006; Covelo *et al.* 2007; Lu and Xu 2009). The current findings agree with the wider literature as it appears that weakly associated Zn was decreased to a greater extent than Cd by either precipitation of Fe/Mn oxy-hydroxides or stable organic compounds, but not to the same magnitude as Pb.

Significant differences in the oxidisable and residual fractions were observed between the high SCG treatment and the other treatment groups, with approximately 17% reduction in the proportion of Zn present in the residual fraction and a similar increase in the percentage of Zn present in the organic fraction. This suggests that there is a removal of Zn from the residual phase and incorporation into the organic fraction, potentially indicating dissolution of mineral forms of Zn and the subsequent incorporation into stable organic matter or sulphides. Such a significant change in these chemically and kinetically resilient fractions is unexpected as mineral phases would be expected to release Zn slowly over long periods of time and the environmental conditions in the column experiment were relatively benign.

However, it is possible that the increased weathering of the mineral phase was due to microbial processes, which induced mineral degradation via bio-erosion, increasing mineral surface areas and chemical dissolution by organic acids (Berthelin 1988; Lian *et al.* 2008; Sokolova 2011). Several investigations have reported the mobilisation of trace elements via microbial degradation and the subsequent fixation of the mobilised elements into complex organic structures (Quantin *et al.* 2001; Rogers and Bennett 2004; Mailloux *et al.* 2009; Becerra-Castro *et al.* 2013), which would give a plausible explanation to the findings in this investigation considering that SCG had the highest peaks of respiration and microbial biomass (sections 6.4.1.2 and 6.4.7). Furthermore, the similar findings for Cd give more weight to this proposal, as Cd and Zn were found to be tightly associated in terms of chemical behaviour and probably geo-chemical origin in the Tyndrum tailings.

Concentrations of weakly associated Zn remain significant in the tailing matrix of all amended tailings, with approximately 5,000 mg kg⁻¹ remaining in this fraction, indicating the limited capacity of using these organic amendments to immobilise Zn in the Tyndrum tailings.

Considering the LMWOA-Zn from the Le Bleyard group, SCG and high applications of SM and GWC were found to have the largest effect on reducing this fraction of Zn, producing moderate reductions of up to 12% as compared to

the un-amended columns. The findings of Zn decrease are likely similar to the outputs discussed for the Tyndrum group, with the potential immobilisation due to complexation with OM or co-precipitation with Mn/Fe oxy-hydroxides, although as discussed for Pb the former may be more likely due to the low potential for PTE-Fe/Mn interaction in an alkaline tailing environment. Overall it appears that organic amendments with either more available OM (SCG or SM) or a more humified nature (GWC) have the largest capacity to sequester Zn in the calcareous Le Bleymard tailings. Cattle manure was not found to be as effective, although the reasons for this are not clear. It may be possible that the organic ligands in CM do not provide as strong binding mechanisms as the other amendments, or that the types of OM found in this material are less effective in stabilising Zn.

6.7.6 Section Conclusions

The investigation of the solid phase extractable PTE in the amended columns have highlighted several findings relating to the immobilisation of PTE and changes in fractionation.

In both mine tailing groups, it appears that there is a hierarchical system of preferential PTE binding and immobilisation to organic ligands or Fe/Mn oxy-hydroxides, in the order of $Pb > Zn > Cd$. Overall it appears that the Pb has the greatest affinity for these available sites and so was removed to the largest extent in comparison to the other elements. Despite the substantial concentrations of Pb remaining in the LMWOA and weakly associated fractions, this demonstrates that the incorporation of organic amendments has a positive effect on the removal of Pb from these fractions, probably limiting the environmental impact to some degree if carbonate associated Pb undergo significant dissolution. Conversely, the considerable baseline concentration of Pb in the tailings may have saturated the binding capacity of the amended tailings and therefore the addition of OM did not have as a significant effect on the immobilisation of Zn or Cd.

This follows the wider literature, suggesting that competitive effects between elements will create displacement and possibly induce increased mobilisation of

Cd and Zn, whose affinity for binding sites are not as strong (Alloway 2012). An increase in mobilisation however did not occur in the most labile fractions assessed, suggesting that the incorporation of OM into the tailings of Le Bleymard and Tyndrum would not exacerbate elemental mobility, but would not reduce it significantly either. Therefore, the immobilisation effect of organic amendments in the Tyndrum tailings to Zn and Cd will be compounded by the elevated concentrations of Pb.

Further findings also suggests that Mn had an important role in the sequestration of Pb in the Tyndrum tailings (and possibly the tailings from Le Bleymard), with a shift in the proportion of Pb from the weakly associated fraction into the reducible pool, subsequently reducing the environmental mobility of Pb. This was seen to the greatest extent in SCG, which incidentally had the highest concentrations of water-extractable Mn as compared to other amendments. Interestingly, the application of SCG also appeared to induce a microbially-driven change in the residual and oxidisable fractions of Cd and Zn. This could have significant consequences in the long-term, as microbial activities may accelerate the weathering of large pools of environmentally negligible PTE which may then be released in a more mobile form. Similar reactions may be occurring in the Le Bleymard tailings, however, due to the inability to apply the BCR procedure to the tailings this remains unknown.

Considering the capacity to reduce PTE mobility in the solid phase it appears that SCG, GWC and high applications of SM are the most suitable for both tailing sites in consideration for their ability to reduce Pb in the weakly associated and LMWOA phase. Overall the main findings of this section are as follows:

1. A hierarchy of PTE immobilisation is apparent, following the trend of $Pb > Zn > Cd$.
2. A limited immobilisation of weakly bound PTE is created upon incorporation of organic amendments.
3. The immobilisation of Cd and Zn is confounded by the presence of Pb.

4. Mn has a significant impact on the immobilisation of Pb in the Tyndrum tailings.
5. Microbial degradation of Cd-Zn mineral phases is apparent in the Tyndrum tailings and may increase PTE mobility in the future.

6.8 Chapter Conclusions

From the three key sections in this chapter it is clear that the incorporation of organic amendments into the tailings sourced from Le Bleymard and Tyndrum produce a wide range of environmental changes. Substantially different impacts were observed in terms of nutrient cycling, microbial activity, PTE leaching and PTE fractionation, both between types and application rates of organic amendments and between the tailing material.

In summary, when organic amendments are applied to the Le Bleymard tailings the presence of elevated Ca^{2+} and Mg^{2+} and a pH of > 8 accelerate the rates of nitrification, promote the immobilisation of soluble organic C, water-soluble P and buffer the potential increase of leaching of DOC mobilised PTE. Despite the decreases in nutrient availability the incorporation of organic amendments increased total reserves of N and improved microbial processes in the tailing material. Furthermore, the addition of organic amendments appeared to reduce the pH of collected leachates and the solid phase material, as well as reducing weakly associated Pb in the solid phase and provide some measure of immobilisation of Zn.

In contrast, the incorporation of amendments into the Tyndrum tailings did not promote the immediate nitrification of introduced N, but increased leachate and solid phase pH, enhanced the concentrations of water-soluble P, TN/TC and improved microbial functioning. Moreover, a general increase in leachate Pb was observed, alongside a concurrent decrease in Cd and Zn. Finally, the fractionation of Pb and Zn was changed upon amendment introduction, promoting the immobilisation of Pb, and to a lesser extent Zn.

Some generalised statements can be made from the chapter on the effects of organic amendment introduction to the two tailings:

1. The incorporation of organic amendments with high C:N ratios may be desirable to limit nutrient loss from tailing systems.
2. The leaching of Zn and Cd are mainly controlled by pH.
3. Weakly associated Pb can be reduced by the addition of organic amendments.
4. The addition of more humified, stable materials such as GWC reduce PTE leaching and enhance Pb immobilisation but increase N loss via NO_3^- and do not promote microbial communities as effectively as more active C sources.

However, it is clear that the source and characteristics of organic amendments can prompt contrasting changes in the tailing environment, and importantly, the properties of the tailings have strong controlling factors on observed environmental changes in the tailings. Therefore, despite the parallels identified it is difficult to predict the effects that organic amendments will have on mine tailings.

This chapter has contributed to our knowledge of observed environmental changes that occur when organic amendments are introduced into contrasting mine tailings. Significant findings include the identification of key processes that promote and suppress the movement of PTE within the aqueous and solid phases, the interactions of tailing characteristics and organic amendments on nutrient availability and speciation and finally, a greater understanding of the holistic effects of adding organic amendments to mine tailings. Furthermore, this work highlights that the usefulness of specific organic amendments in the remediation of Pb/Zn mine tailings must be treated with caution, as divergent differences have been shown depending on the underlying properties of the material to be remediated. Finally, the chapter contributes to the wider scientific literature on the use of organic amendments in the remediation of contaminated land and provides empirical evidence that may aid in the selection of waste materials in future projects.

An overview of findings for each tailing group and the incorporated amendments are presented in (Table 6-8 and Table 6-9).

Table 6-8. Summary table of findings for organic amendments incorporated into the Tyndrum tailings. Bold formatting indicates amendment with the greatest effect.

Parameter	CM	SM	GWC	SCG
pH	Increased in leachate and solid phase	Increased in leachate and solid phase	Increased in leachate and solid phase	Increased in leachate
Total Carbon	Increased	Increased	Increased	Increased
Total Nitrogen	No Change	Increased	Increased	Increased
Soil Respiration	Increased	Increased	No Change	Increased
Microbial Biomass	Increased	Increased	No Change	Increased
PTE Leaching	High Pb Low Cd/Zn	High Pb Low Cd/Zn	Decreased all	Moderate Pb Low Cd/Zn
Nutrient Leaching	High increase	High increase	High increase	Low increase
Plant available N	Increased	Increased	Increased	Decreased
Plant available P	Increased	Increased	Increased	Increased
‘Mobile’ PTE	Decreased	Decreased	Decreased	Decreased

Table 6-9. Summary table of findings for organic amendments incorporated into the Bleymard tailings. Bold formatting indicates amendment with the greatest effect.

Parameter	CM	SM	GWC	SCG
pH	Decreased in leachate and solid phase	Decreased in leachate and solid phase	No Change	Decreased in leachate and solid phase
Total Carbon	Increased	Increased	Increased	Increased
Total Nitrogen	Increased	Increased	Increased	Increased
Soil Respiration	Increased	Increased	No Change	Increased
Microbial Biomass	Increased	Increased	No Change	Increased
PTE Leaching	Increased Pb, Cd and Zn	Increased Pb, Cd and Zn	No Change	Increased Pb, Cd and Zn
Nutrient Leaching	High increase	High increase	High increase	Low increase
Plant available N	No Change	Increased	No Change	Decreased
Plant available P	Increased	Increased	Increased	Increased
‘Mobile’ PTE	Decreased	Decreased	Decreased	Decreased

Chapter 7. Field Trial of Organic Amendment Assisted Remediation

7.1 Introduction

This final chapter focuses on a seven-month field trial involving the incorporation of selected organic amendments and the growth of *Agrostis capillaris* and *Festuca ovina* in treatment plots spread across the mine tailing areas at each of Tyndrum and Le Bleymard. Monitoring was carried out at regular intervals throughout the investigation and upon conclusion vegetation and tailing material were sampled and assessed for a range of physical and chemical properties.

This chapter is divided into three parts: the first considering the findings at Tyndrum; the second explores the results from Le Bleymard; the final section compares the findings at each site and summarises the work, highlighting the key findings and scientific contributions.

7.2 Rationale and Overview

This chapter was essential in validating findings and the hypotheses established in Chapters 5 and 6, by bringing the concepts from laboratory to a field environment and assessing the suitability of proposed remedial approaches under real settings. There is a strong need for more field trials to be completed to assess the suitability of phytoremediation of mine tailings, a criticism that has been repeatedly voiced from the scientific community and has been attributed to the lack of uptake by industry (Robinson *et al.* 2015). Finally, direct comparisons of similar remedial techniques across contrasting mining sites are lacking, limiting the available knowledge on the important underlying variables that may affect the success of phytoremediation projects.

This chapter presents a novel investigation of phytostabilisation on two contrasting mine tailings by comparing site specific characteristics and a generic organic amendment with the use of previously highlighted phytostabilisation species. The chapter highlights key tailing characteristics that can influence

phytoremediation success as well as identifying other environmental conditions that are important in the remediation and assessment of phytoremediation of abandoned Pb/Zn mines. To the best of the author's knowledge this is the first investigation of its kind that attempts to compare two diverse mine tailings and harmonise the findings.

7.3 Methods and Statistical Approaches

7.3.1 Methods

A full overview of the experiment can be found in Chapter 3, section 3.5.

Trial plots were established in Le Bleymard and Tyndrum by mixing the relevant amendment into designated plot areas (area 0.13 m², depth 30 cm), replicated randomly five (Le Bleymard) and six (Tyndrum) times across the tailing area at each mine. The plots were contained using soil gas sampling chambers to provide facilities for gas measurement and to outline plot boundaries. At each plot location three replications of the experimental conditions were applied, untreated tailings, a GWC amendment and a site-specific amendment (SCG or CM). The amendments were applied at a rate of 200 t ha⁻¹, based on findings from Chapter 6. The amendments were thoroughly mixed into the tailing substrate to a depth of 30 cm and Rhizon® samplers installed to collect pore water. Overall, six replications of each treatment were installed at Tyndrum and five at Le Bleymard (Table 7-1).

Table 7-1. Overview of treatments used at the Tyndrum and Le Bleymard field trial investigations. Note: 5 replications of each amendment and an untreated control were included in the Le Bleymard site and 6 replicates for the Tyndrum site respectively.

Amendment	Le Bleymard	Tyndrum
Untreated control	✓	✓
Green Waste Compost (GWC)	✓	✓
Spent coffee grounds (SCG)	✗	✓
Cattle manure (CM)	✓	✗

Sampling was conducted monthly at Tyndrum for pore water, gas and vegetative development, and every second month at Le Bleymard. No gas or pore water sampling was conducted at Le Bleymard due to logistical difficulties and sample preservation. Destructive sampling occurred upon the conclusion of the investigation, on the 28th of September 2017 and 23rd of October 2017 for Tyndrum and Le Bleymard respectively. A tailing core was taken from each trial pit and placed in a clean labelled polyethylene bag. Vegetation was removed and prepared for analysis following section 3.6.4. Within 3 hours of core sampling and placed in separate bags. A range of chemical and physical parameters were applied to identify differences between treatments and plot locations (*Chapter 3, section 3.5*).

7.3.2 Statistical Approaches

Analysis of variance (ANOVA and Analysis of Means) and linear regression were considered the most appropriate tools for this investigation to allow for several important parameters to be considered at once. Statistical measurements were completed using Minitab v. 18 and graphical outputs produced in Origin 2016. Significance testing was completed at the 0.05 confidence level for all tests.

7.4 Tyndrum Field Trial

7.4.1 BCR-Sequential Extraction of the Solid Phase

7.4.1.1 Cd

The distribution of Cd among the operationally defined phases found in the tailings material collected from the amended and control plots is presented in Figure 7-1. Analysis of means showed that total concentrations of Cd were significantly higher in the SCG treatments as compared to either the GWC or the un-treated tailings ($p = <0.05$). Contrastingly, the application of SCG and GWC significantly increased the proportions of Cd in the reducible fractions by ~11% and ~16%, respectively. Amendment with SCG led to a lower proportion of Cd in the weakly associated fraction (~15% less than for the control) as compared with the proportion of Cd in this fraction after amendment with GWC (~11%

lower than the control). No significant differences in the residual or oxidisable fractions of Cd were observed across any treatments.

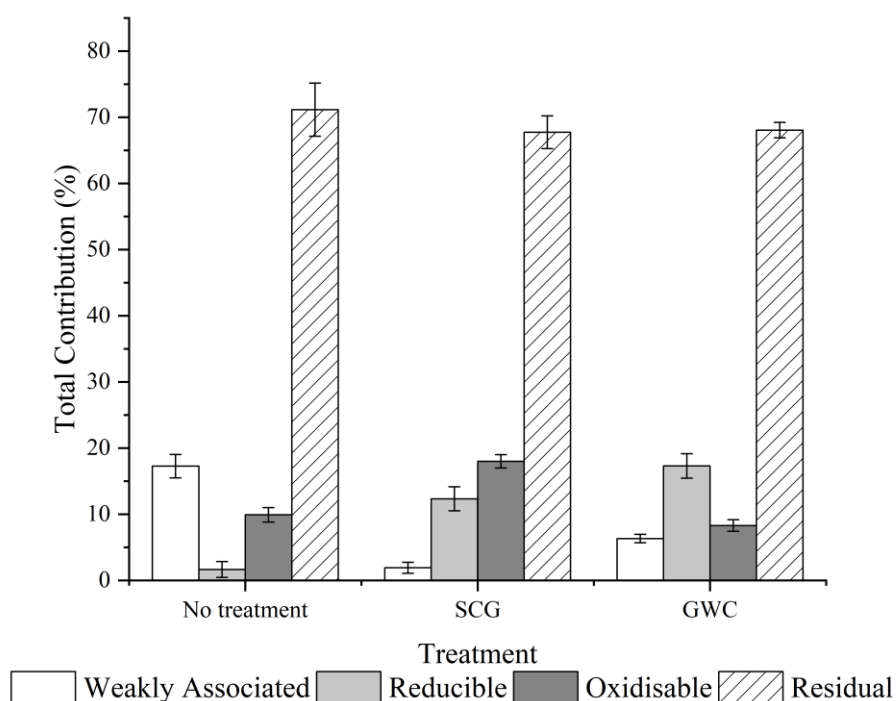


Figure 7-1. Relative proportions of BCR-extractable Cd from the amended mine tailings sourced from Tyndrum.

7.4.1.2 Pb

Proportional fractionation data for BCR-extracted Pb are displayed in Figure 7-2. Weakly associated Pb was significantly reduced upon the application of SCG and GWC, with a mean reduction of ~37% and ~29%, respectively ($p < 0.05$). The proportion of Pb extracted in the reducible fraction was significantly increased by both GWC (~38%) and SCG treatments (~20%) relative to the un-amended control. Spent coffee grounds also significantly increased Pb held in the oxidisable fraction as compared to the un-amended tailings by ~17%, whereas GWC application significantly lowered the proportion of Pb in the

oxidisable fraction by ~9% relative to the un-treated tailings ($p = <0.05$). The proportion of Pb in the residual fraction was not significantly different across treatments and this fraction also had the lowest proportions of Pb across all fractions ($p = >0.05$).

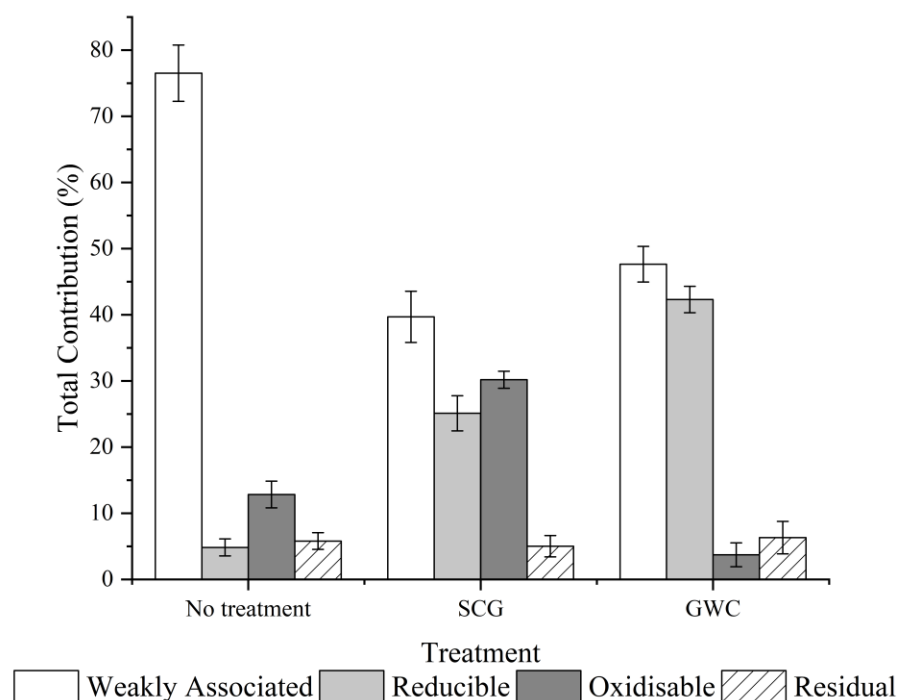


Figure 7-2. Relative proportions of BCR-extractable Pb from the amended mine tailings sourced from Tyndrum.

7.4.1.3 Zn

BCR-extractable Zn is displayed in Figure 7-3. The results showed that Zn was significantly higher in the residual fractions across all treatments, with the reducible fraction containing the lowest concentrations of Zn within the tailing matrix. Spent coffee grounds had a significant effect on the total concentration of Zn within the tailings, with this treatment group increasing total Zn in comparison to the un-treated and GWC applications. However, no significant differences were observed between the four-step sum and the separate total extraction ($p = >0.05$). No significant differences were observed between other fractions or groups, despite increases in the proportion of Zn in the oxidisable

fraction following GWC treatment and a greater percentage increase in the reducible fraction in the SCG amended tailings.

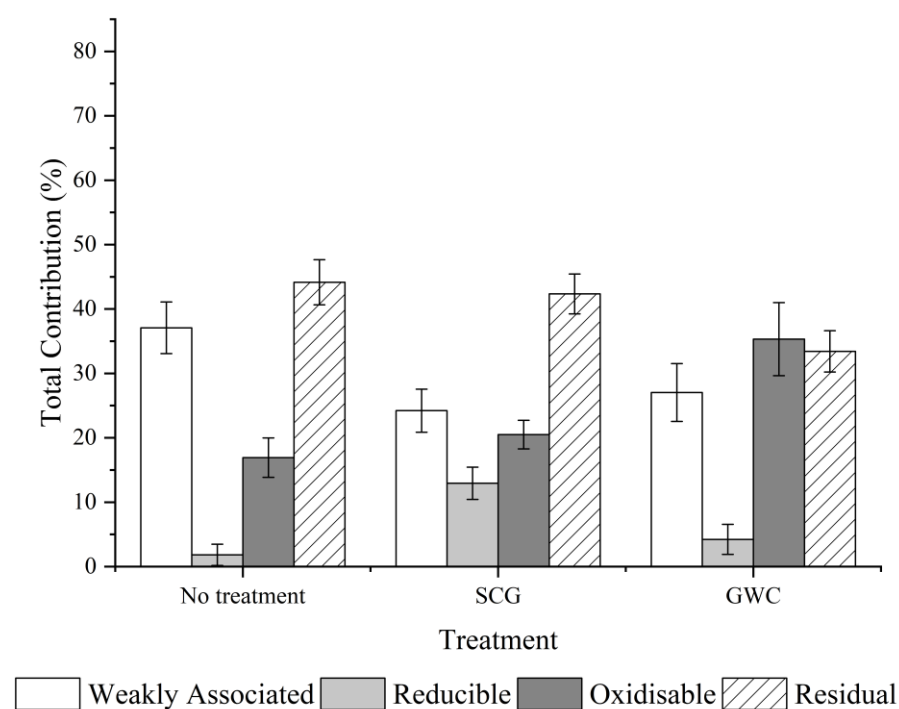


Figure 7-3. Relative proportions of BCR-extractable Zn from the Tyndrum field trials.

7.4.2 Calcium Chloride – Extractable Cd, Pb and Zn.

The CaCl_2 – extractable Cd concentrations obtained for the treated and untreated tailings were not significantly different (Figure 7-4a). However, significant differences were observed between plot locations (Figure 7-4b), with plot 5 having significantly greater CaCl_2 -extractable Cd in comparison with plots 1, 2, 4 and 6 ($p = <0.05$).

Similar findings were observed for Pb (Figure 7-4c) and Zn (Figure 7-4e), with no significant differences in CaCl_2 – extractable elemental concentrations when considering treatment groups. However, higher CaCl_2 -extractable Pb concentrations were obtained for plots 2 and 6 as compared to plots 1 and 3 (Figure 7-4d). Similarly to Cd, plot 5 had significantly higher concentrations of CaCl_2 – extractable Zn than plots 1 and 2 (Figure 7-4f).

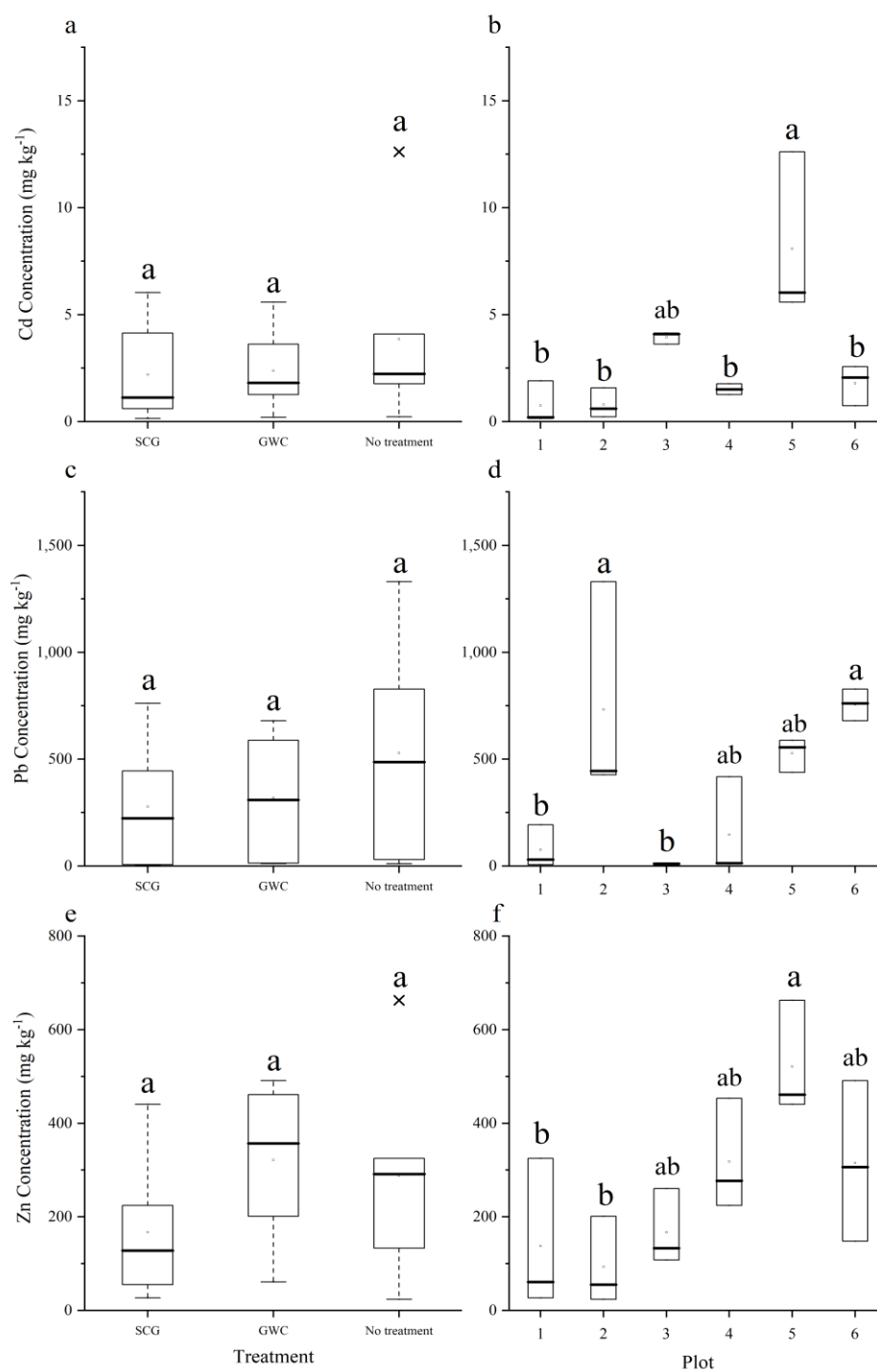


Figure 7-4. Calcium chloride - extractable Cd (a–b), Pb (c–d) and Zn (e–f) concentrations from the Tyndrum field site by treatment (Left) and by plot location (Right). Means that do not share a letter are significantly different for aerial and below ground components respectively at a value of ($\alpha = 0.05$, $n = 6$).

7.4.3 Plant Growth, Cover and PTE Uptake

7.4.3.1 Vegetation Height and Biomass

Vegetation height and percentage cover was significantly higher in the SCG treatments as compared to the un-treated and GWC amended groups (Figure 7-5). Vegetation across all treatments was dominated by *A.capillaris*, with only a few shoots of *F.ovina* visually identified during this investigation.

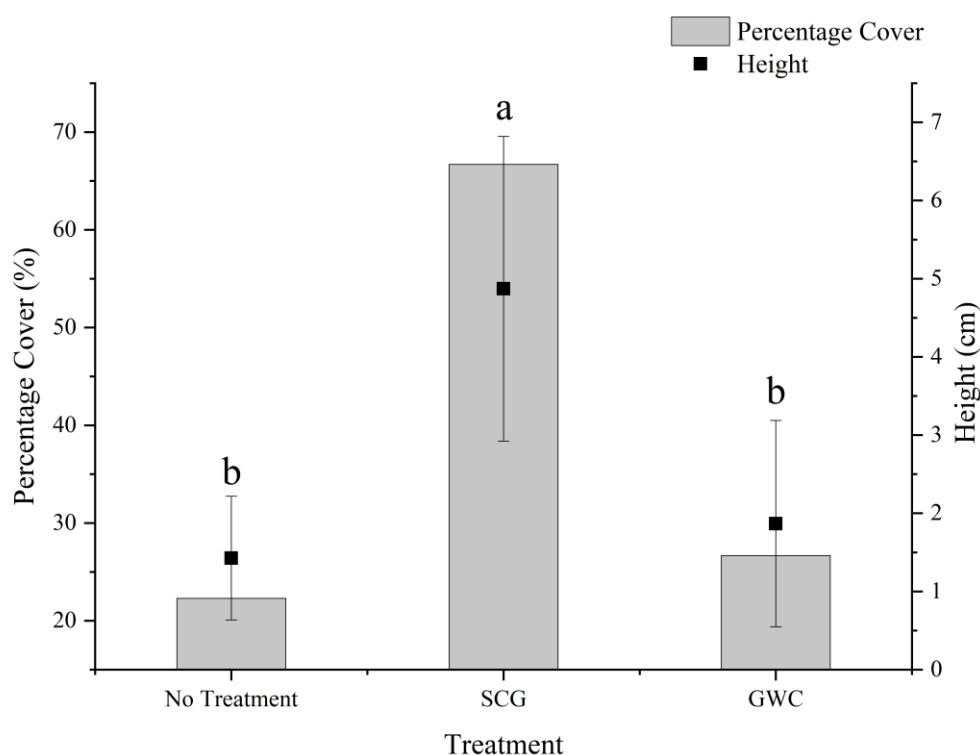


Figure 7-5. Vegetation height and surface percentage cover of treated and un-amended tailings at the Tyndrum mine. Means that do not share a letter are significantly different for aerial and below ground components respectively at a value of ($\alpha = 0.05$, $n = 6$).

Shoot and root biomasses of vegetation from the Tyndrum tailings were significantly higher in the SCG and GWC treatments as compared with the un-amended tailings (Figure 7-6) ($p < 0.05$). Shoot biomass in SCG treated tailings were significantly higher than for the GWC group, but no significant differences were observed between root biomass between GWC and SCG ($p > 0.05$).

There was a noticeable difference in the distribution of shoot and root biomass at the different plot locations (Appendix 9) and, in particular, it appeared that the

tailing material at plot 5 had an inhibitory effect on the biomass for all treatment groups at this location. Moreover, no growth for the un-treated tailings were observed at this location.

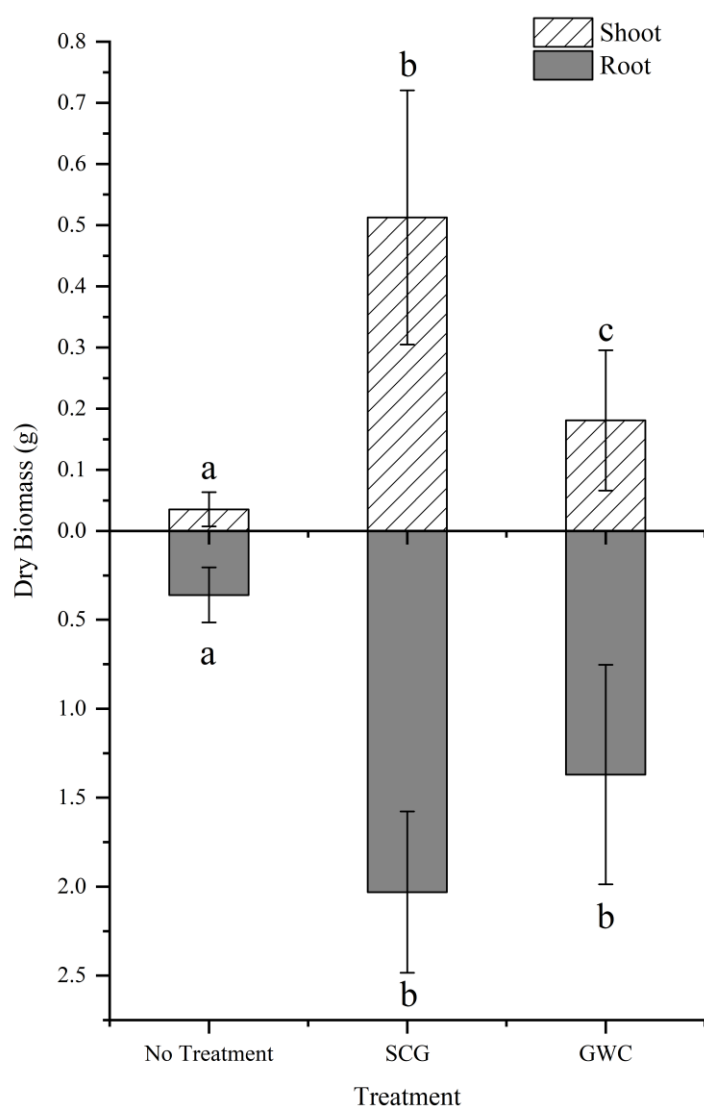


Figure 7-6. Shoot and root biomass of treated and un-amended tailings at the Tyndrum site. Means that do not share a letter are significantly different for aerial and below ground components respectively at a value of ($\alpha = 0.05$, $n = 6$).

7.4.3.2 PTE Concentrations in the Shoots and Roots of Plants.

Cd

Cadmium concentrations in the root and shoots of the vegetation for each treatment group are presented in Figure 7-7. Cadmium concentrations were not

significantly different across treatments in terms of uptake into root systems or translocation into the aerial compartments in the plant, and were approximately 3 mg kg^{-1} in all treatments.

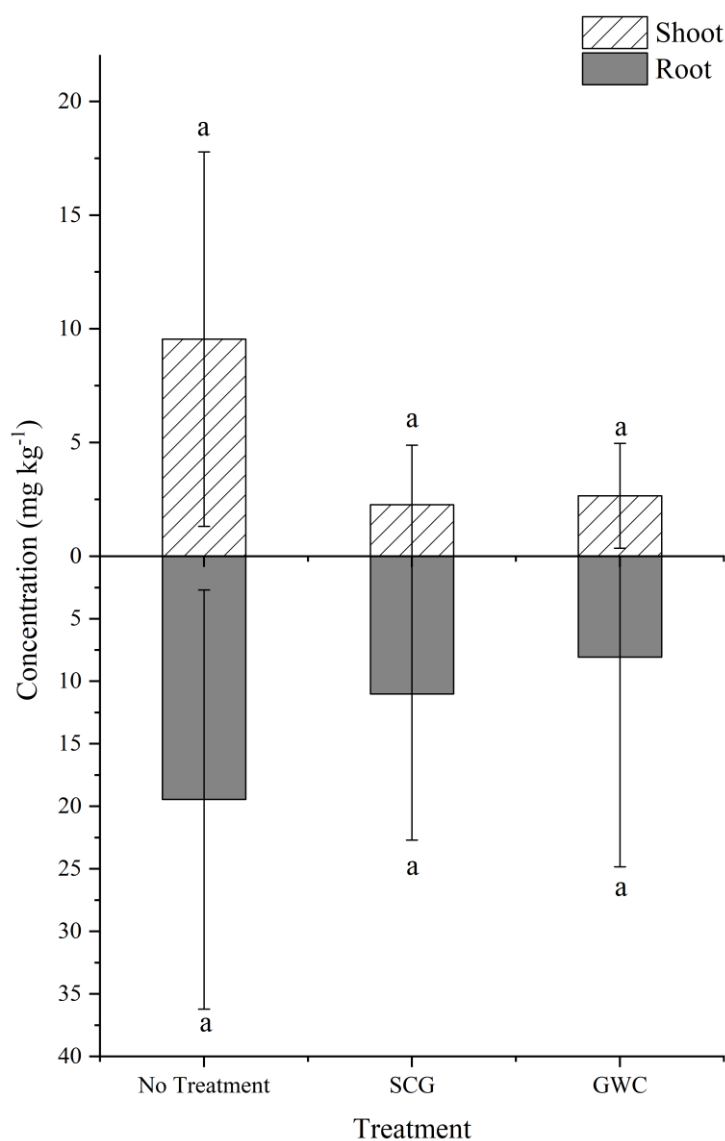


Figure 7-7. Cadmium concentrations in the roots and shoots of the untreated and amended tailings at the Tyndrum site. Means that do not share a letter are significantly different for aerial and below ground components respectively at a value of ($\alpha = 0.05$, $n = 6$).

Pb

Lead uptake into the roots of the sampled vegetation was over $5,000 \text{ mg kg}^{-1}$ for all treatment groups, with no significant differences observed between groups (Figure 7-8). Translocation of Pb to the shoots was observed to be significantly

lower in vegetation amended with GWC in comparison to the un-treated control material, with no significant differences observed between SCG and the control. For all treatments Pb concentration in the shoots exceeded 1,000 mg kg⁻¹.

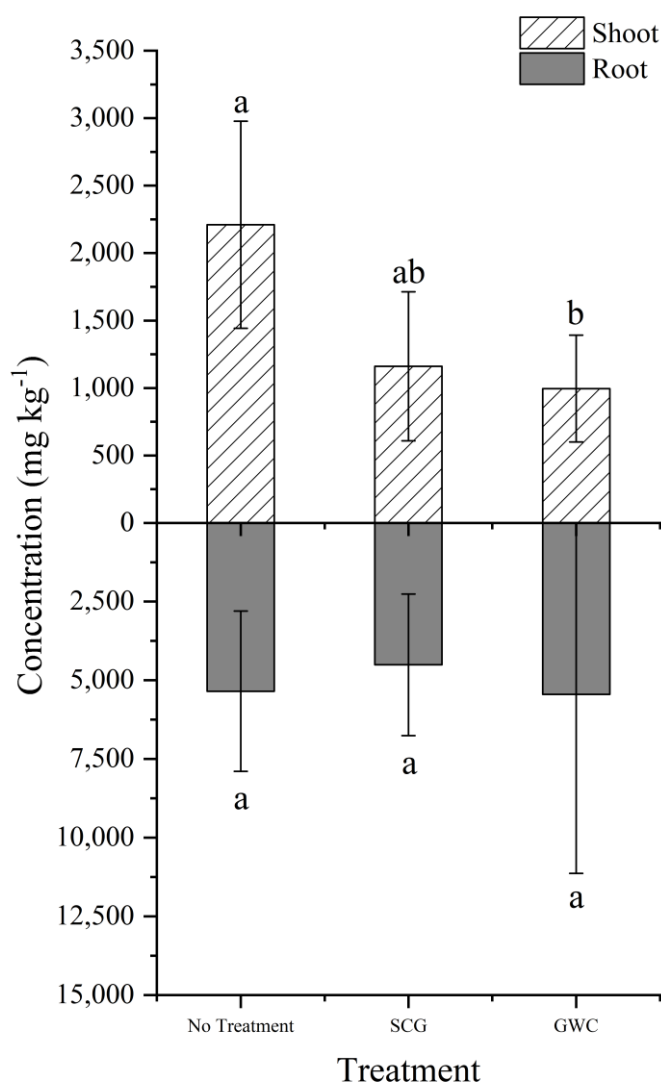


Figure 7-8. Lead concentrations in the roots and shoots of the untreated and amended tailings at the Tyndrum site. Means that do not share a letter are significantly different for aerial and below ground components respectively at a value of ($\alpha = 0.05$, $n = 6$).

Zn

The absorption of Zn into plant roots and the translocation into shoots was not significantly different between treatment groups (Figure 7-9). Mean Zn concentrations in the shoots ranged between 2,000 – 3,600 mg kg⁻¹ and between 3,800 – 5,600 mg kg⁻¹ in the roots.

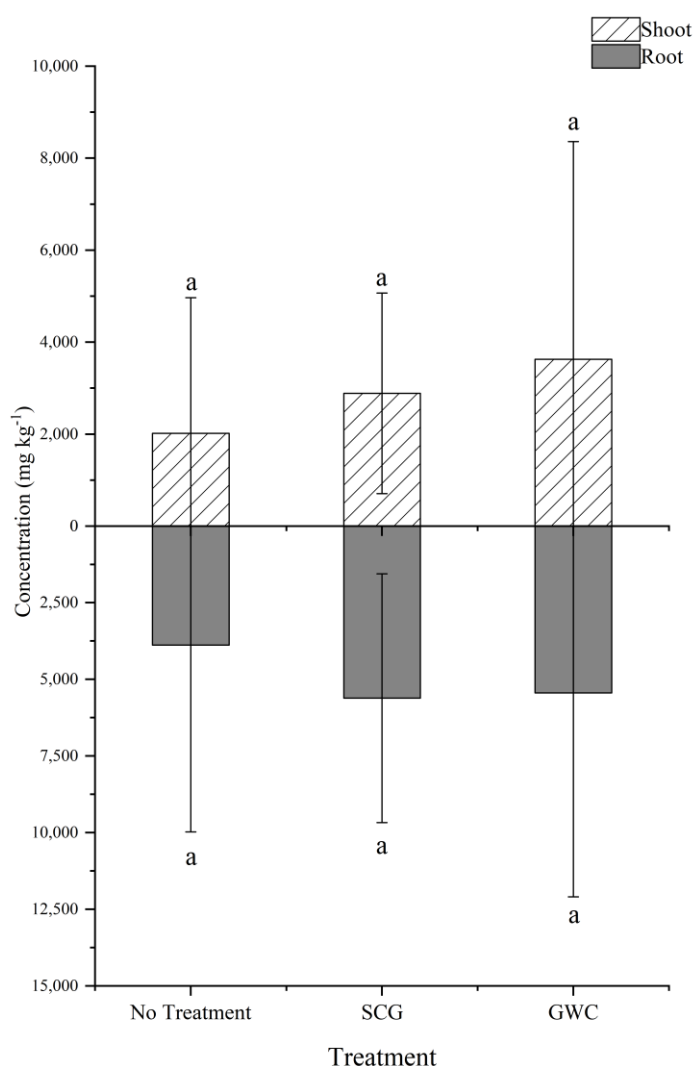


Figure 7-9. Zinc concentrations in the roots and shoots of the untreated and amended tailings at the Tyndrum site. Means that do not share a letter are significantly different for aerial and below ground components respectively at a value of ($\alpha = 0.05$, $n = 6$).

7.4.3.2 Translocation and Bioaccumulation Factors

Bioaccumulation coefficients (BC) and translocation factors (TF) (Chapter 3) for each treatment and element are presented in (Table 7-2). Cadmium BC were significantly lower for vegetation growing in the GWC and SCG amended tailings as compared to the un-treated control. Similarly, BC and TF for Pb were also significantly lower in the treated tailings in comparison to the un-amended tailings. In contrast, the BC for Zn was highest in vegetation growing in GWC treated tailings, with no significant differences observed between the SCG and

un-treated groups. Translocation factors for Zn were not significantly different across treatment groups.

Bioaccumulation coefficients were significantly different for each element and in the order of $Zn > Pb > Cd$ over all treatments. Translocation factors were significantly higher for Zn and statistically similar for Pb and Cd across all treatments.

Table 7-2. Bioaccumulation coefficients (BC) and translocation factors (TF) for Cd, Pb and Zn in sampled vegetation by treatment at the Tyndrum mine tailings. Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$) and are highlighted in bold. $n = 6$

Element	Treatment	BC	TF
Cd	SCG	0.22 (± 0.05) ^a	0.28 (± 0.17) ^a
Cd	GWC	0.22 (± 0.06) ^a	0.31 (± 0.14) ^a
Cd	No Treatment	0.38 (± 0.04)^b	0.44 (± 0.09) ^a
Pb	SCG	0.29 (± 0.04) ^a	0.26 (± 0.06) ^a
Pb	GWC	0.37 (± 0.07) ^a	0.22 (± 0.07) ^a
Pb	No Treatment	0.62 (± 0.1)^b	0.43 (± 0.1)^b
Zn	SCG	0.64 (± 0.07) ^a	0.54 (± 0.16) ^a
Zn	GWC	0.92 (± 0.07)^b	0.61 (± 0.18) ^a
Zn	No Treatment	0.65 (± 0.11) ^a	0.47 (± 0.36) ^a

7.4.4 Nitrogen, Carbon, Water Soluble P Concentrations and pH

7.4.4.1 Total Nitrogen and NO_3^- -N / NH_4^+ -N Concentrations

Total nitrogen was significantly elevated in both GWC and SCG treatments as compared to the un-amended tailings, with an increase of approximately 0.14 and 0.18 % respectively (Table 7-3). Furthermore, SCG significantly increased the concentrations of extractable NO_3^- and NH_4^+ in comparison to both the GWC treatments and the un-amended control. No significant differences in NO_3^- and NH_4^+ were observed between the GWC and un-treated tailings.

Table 7-3. Measured N parameters in the treated and untreated mine tailings at Tyndrum.
Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$),
highlighted in bold. n = 6.

Treatment	Total N (%)	NO ₃ ⁻ (mg kg ⁻¹)	NH ₄ ⁺ (mg kg ⁻¹)
SCG	0.20 (± 0.06) ^a	8.95 (± 2.6)^a	6.42 (± 1.5)^a
GWC	0.16 (± 0.08) ^a	2.51 (± 3.1) ^b	2.17 (± 1.0) ^b
No Treatment	0.03 (± 0.03)^b	0.21 (± 0.15) ^b	0.84 (± 0.66) ^b

7.4.4.2 Total Carbon and Hot and Cold Water Extractable Carbon

Total C was significantly higher in both GWC and SCG-treated tailing plots as compared with the un-treated control (Table 7-4). Cold water extractable C was significantly higher for the GWC-treated tailings in comparison with the un-amended tailings and the SCG, with the HWE C highest in GWC, followed by the SCG treatment.

Table 7-4. Measured N parameters in the treated and untreated mine tailings at Tyndrum.
CWE C = Cold water extractable carbon, HWE C = Hot water extractable carbon. Means that do
not share the same letter are significantly different at a value of ($\alpha = 0.05$), highlighted in bold.
n = 6.

Treatment	Total C (%)	CWE C (mg kg ⁻¹)	HWE C (mg kg ⁻¹)
SCG	3.27 (± 0.77) ^a	77.6 (± 16.4) ^b	386 (± 129) ^c
GWC	3.32 (± 1.6) ^a	203 (± 53)^a	823 (± 105) ^a
No Treatment	0.75 (± 0.67)^b	71.9 (± 8.6) ^b	218 (± 25.9) ^b

7.4.4.2 Water Extractable P

Water extractable P in the SCG treatments (15.32 ± 8.6 mg kg⁻¹) was significantly higher as compared to the un-amended control (2.88 ± 2.1 mg kg⁻¹), but not in comparison to GWC treatments (9.91 ± 3.82 mg kg⁻¹). Green waste compost did not have significantly different concentrations of water-soluble P as compared to the un-treated tailings ($p = >0.05$).

7.4.4.2 Tailing pH and Particle Size

The geometric mean pH of the tailings were not significantly different across treatment groups (~ 6.5), however, significant differences were observed across

plot locations (Figure 7-10), with the highest pH found in plot 3 and the lowest in plot 2.

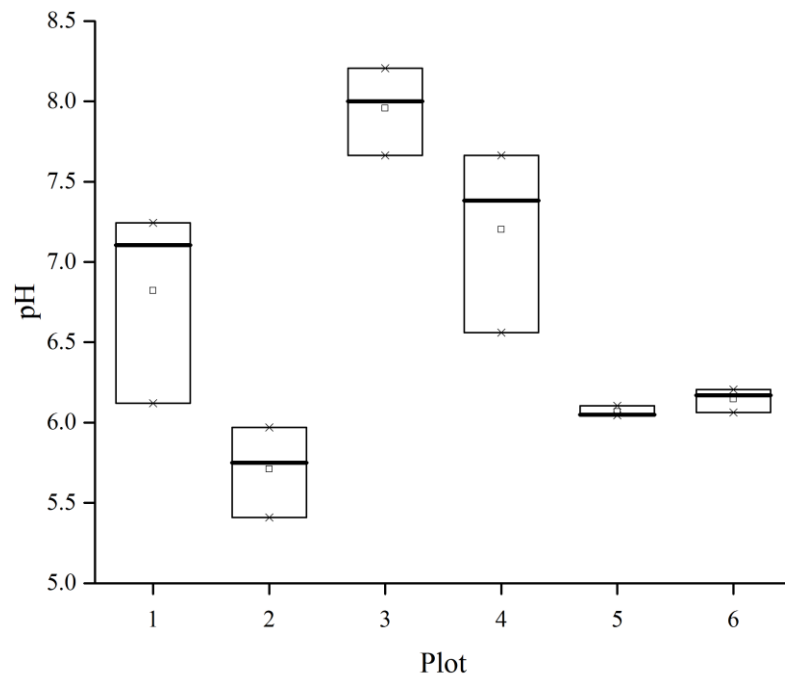


Figure 7-10. Plot locations and geometric mean of pH in the Tyndrum mine tailings. $n = 3$.

The median particle size across plot sites was generally $<200 \mu\text{m}$, in the exception of plot 4, where the median particle size was $629 \mu\text{m}$ (Figure 7-11).

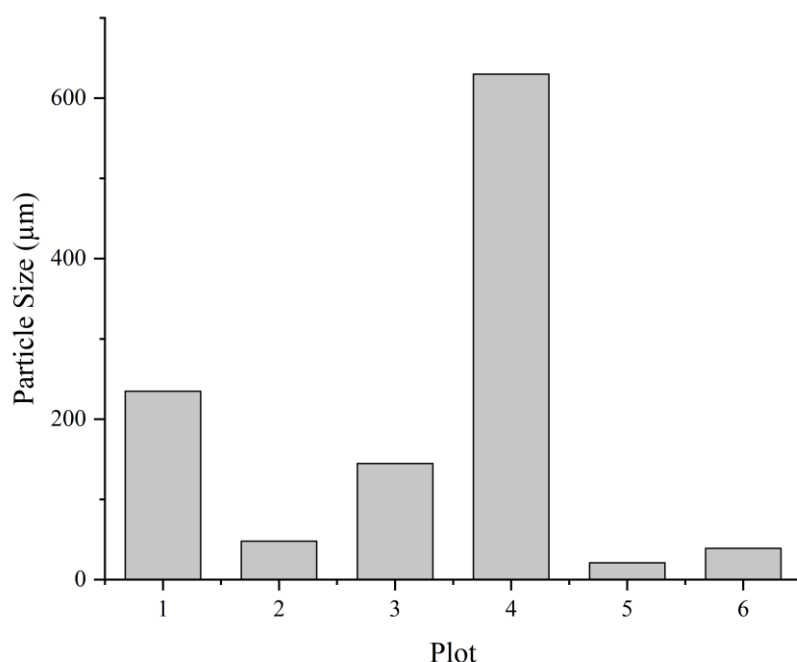


Figure 7-11. Median particle size of tailing material at each plot location, n=3

7.4.5 Prediction of PTE Bioavailability

The concentrations of weakly associated Pb was observed to have strong correlations with plant available and total concentrations of Pb and Zn (Figure 7-12). Calcium chloride extractions were not strongly correlated to plant uptake or total tailing concentrations of Pb, Zn or Cd. The predictive power of PTE uptake using the weakly associated extraction was greater for Pb than Zn, with this fraction accounting for 98% of the total variance of Pb uptake in the sampled vegetation.

Furthermore, neither the concentration of weakly associated Cd nor CaCl_2 extractable Cd concentration predicted plant uptake for this element (Figure 7-12e - f). Combinations of BCR fractions (e.g. BCR-1 + BCR 2) also did not improve the prediction of plant uptake of Cd in the tailings. The Pearson product-moment correlation coefficient for each element can be found in Appendix 7.

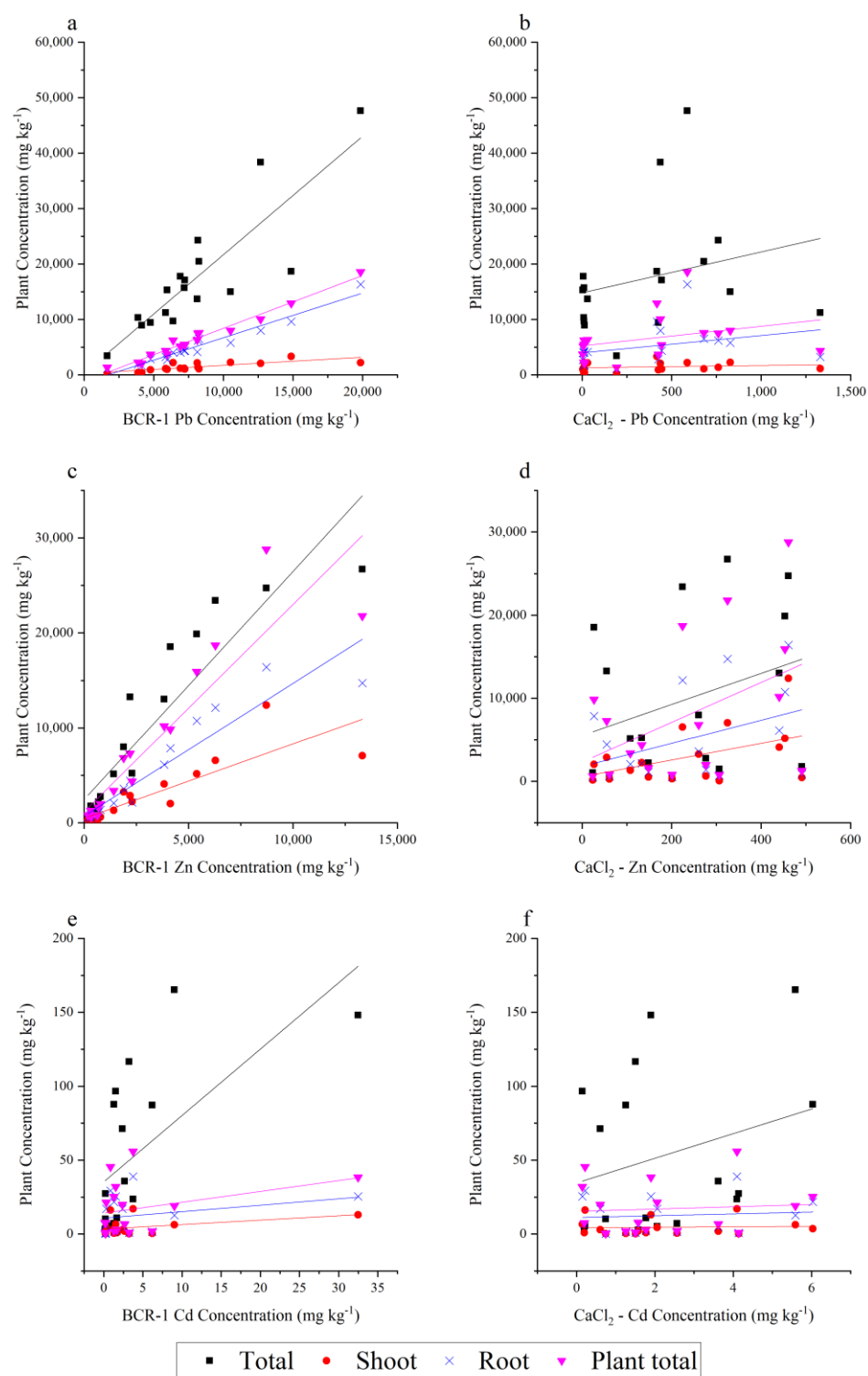


Figure 7-12. Correlations between plant PTE concentrations, total tailing concentrations and BCR-1/ CaCl_2 extractable Pb (a-b), Zn (c-d) and Cd (e-f) at the Tyndrum site.

7.4.6 Discussion of Tyndrum Findings

7.4.6.1 Vegetation Establishment and Tailing Nutrient Content

The application of SCG and GWC increased vegetation development of *A.capillaris* as compared to the un-treated control, with over a two-fold increase in root biomass in both treatments, and an approximately five-fold increase in shoot development in the SCG amended tailings. This suggests that the input of organic amendments and the resulting increases in TN, TC, and extractable pools of carbon aided in supporting greater vegetative development primarily through the provision of plant nutrients. This is also likely to have enhanced a range of other essential tailing properties, such as microbial communities, nutrient cycling systems and tailing physical properties (Basanta *et al.* 2017; Page-Dumroese *et al.* 2018; Santini and Fey 2018). Amendment with SCG increased shoot biomass, percentage cover and vegetation height to a greater extent as compared to the GWC treatments, likely related to the increased concentrations of extractable NO_3^- , NH_4^+ and P in these treatments and the resulting support for plant tissue development (Silva and Uchida 2000; Storey 2006). It is possible that the reported leaching of NO_3^- from GWC treatments in Chapter 6 also occurred in the field trials, leading to a loss of plant available N over time, and importantly, before seedling germination and vegetative establishment occurred. Conversely, the immobilising impact to labile N demonstrated in the short term by SCG in Chapter 6 does not appear to be permanent, as concentrations of NO_3^- and NH_4^+ were significantly elevated in these treatments, supporting the concept of initial immobilisation and thereafter a slower release of plant available N in SCG amended soils (Wakasawa *et al.* 1998; Yamane *et al.* 2014).

Vegetation parameters varied with plot location (Appendix 9), indicating that the consistency of vegetation establishment across the site is erratic and likely influenced by the intrinsic heterogeneity. For example, plot 5 was noted to generally have lower root and shoot biomasses and percentage cover across all treatments, indicating that underlying characteristics, possibly such as particle size and PTE concentration have a significant impact on the success of vegetative growth (Sheoran *et al.* 2010; Ma *et al.* 2016; Colombi *et al.* 2018). Furthermore, the vegetation was visually found to be dominated by *A.capillaris*,

with only a few observations of *F.ovina* establishment on site. This demonstrates a greater tolerance of *A.capillaris* to the environmental conditions in the mine tailings and highlights the usefulness of this species as a phytoremediation tool. However, uptake of PTE into the roots and translocation into the shoot was a concerning finding in this investigation and is explored in more detail in sections 7.4.6.2 – 7.4.6.3.

The absence of *F.ovina* across all trial plots highlights the need for careful plant selection to be applied for potential phytoremediation of the Tyndrum tailings. The absence of the species may be partly attributable to exposure to the elevated concentrations of Pb, Zn and Cd found in the tailings, leading to inhospitable environmental conditions for seedling growth and establishment, despite expected support from organic amendment inputs. Referring back to the findings in Chapter 5 (5.5.5) it is possible that the observation of higher translocation factors of Pb in this species in comparison to *A.capillaris* (Table 5-3) were a controlling factor, and generated significant oxidative stress in sensitive plant tissues. Furthermore, plant tissue concentrations of Pb and Zn in *A.capillaris* in the field trials were several orders of magnitude higher than those tested in Chapter 5, perhaps suggesting that at these concentrations *F.ovina* is not a suitable species for phytoremediation. It is possible that the sowing of tolerant ecotypes may be more appropriate, although this makes the species less favourable for phytoremediation projects as seeds would need to be harvested from relevant populations (Urquhart 1971; Brown and Brinkmann 1992).

Overall, vegetative biomass and surface cover was significantly increased by the addition of both GWC and SCG, indicating that both materials can be useful in potential revegetation efforts of the Tyndrum mine tailings. This is probably due to reduced PTE uptake into plant tissues as discussed in the following section, but also improvements in the tailing environment in terms of vegetative support. In particular, SCG appears to have a greater capacity in accelerating and supporting revegetation, possibly due to a more sustainable release of available N and P into the tailing system in comparison to predicted N losses from the incorporation of GWC.

7.4.6.2 Cd Fractionation and Plant Uptake

The associations of Cd in the solid phase of the treated tailings were significantly altered by the application of GWC and SCG, and potentially to some extent, by the presence of vegetation. Weakly associated Cd was proportionately decreased in the treated tailings as compared to the un-amended tailings, with a resulting shift of Cd into the reducible fraction, suggesting a decrease in bioavailability or chemical lability in the tailing environment. Contrastingly, Cd concentrations in the root and shoots of all treatments were statistically similar, despite clear decreases in BC in the GWC and SCG amended tailings. There was a high degree of variability in the total concentrations and CaCl_2 -extractable Cd, with significant differences observed between plots. This led to high variances in arithmetic models and so the differences in actual Cd uptake and plant tissue concentrations between groups were not statistically different.

However, the relative bioaccumulation of Cd for each treatment was significantly reduced, providing evidence that the amendments suppressed Cd uptake into plants. This has been demonstrated in numerous studies with GWC (Baldwin and Shelton 1999; Hanc *et al.* 2009; Sato *et al.* 2010; Al Mamun *et al.* 2016), but this is the first study where a reduction of Cd uptake into plants has been demonstrated by the application of SCG. A small number of studies have indicated the applicability of SCG in removing aqueous Cd^{2+} from synthesised aqueous media (Azouaou *et al.* 2010; Ayala and Fernández 2018) and in the reduction of Mehlich-3 extractable Cd in soils (Kim *et al.* 2014), with the authors suggesting adsorption onto surfaces as the primary mechanism in the former, and the possible precipitation or complexation with organic ligands in the latter.

In this study, the translocation of Cd was not observed to be significantly different between treatments, indicating that the application of organic amendments does not reduce the risk of Cd accumulation into aerial components. The wider literature indicates that Cd translocation is possibly mediated by Zn transportation mechanisms (Bækgaard *et al.* 2010; Rascio and Navari-Izzo 2011), due to the analogous nature of Cd and Zn and therefore translocation of Cd into shoots is not unexpected in this sense.

In this study, the lower uptake of Cd into plant tissues may be attributable to Cd immobilisation via Fe/Mn hydrous metal oxides (Mahar *et al.* 2015). However, plant uptake of Cd was not predicted by either CaCl_2 – extractable concentrations, or by single, or a combination of the measured variables (Appendix 7). This highlights the difficulty in using single extraction methods to predict Cd uptake, which in this investigation tended to underestimate plant available Cd. This further suggests that although there appears to be a decrease in weakly associated Cd and an increase in reducible Cd, this immobilisation effect does not translate into a decrease in plant uptake. Therefore another mechanism, or combination of variables is probably a controlling factor in the lower BCs observed in these treatment groups for Cd.

Cadmium uptake by plants has been reported to be controlled by pH (Vítková *et al.* 2018), isotopic signatures (Wei *et al.* 2018) genetic predisposition of uptake (Feng *et al.* 2018; Palutoglu *et al.* 2018), and has been suggested to be predicted by the use of several chemical extractants (Novozamsky *et al.* 1993; Feng *et al.* 2005; Meers, Du Laing, *et al.* 2007; Zhang, Liu, *et al.* 2010). However, a recent review by (Kumpiene *et al.* 2017) concluded that bioavailability is highly site specific and thus can be difficult to generalise using chemical parameters, a finding that is supported by this investigation.

Ecological safety threshold values for Cd in vegetation are poorly defined, and often focused on human consumption of crops (Ding *et al.* 2018; Safari *et al.* 2018). However the accumulation of Cd in plant shoots has been reported to have eco-toxicological impacts (Scheirs *et al.* 2006; Aslam *et al.* 2015; Clemens and Caroline 2016) and so the elevated concentrations presented in this study may facilitate the transfer of Cd into food webs if full scale remediation was to occur. This is however considered a low risk due to the small scale of the Tyndrum site, the low likelihood of significant vegetative grazing and potential benefits gained in terms of minimising tailing transportation through the downstream environment.

7.4.6.3 Pb Fractionation and Plant Uptake

The transformation of Pb was significantly altered upon the inclusion of GWC and SCG. Concentrations in the weakly associated Pb decreased by ~30-40% following the addition of these amendments and there was a concomitant increase in Pb concentration in reducible and oxidisable fractions for GWC and SCG, respectively (Figure 7-2). This suggests that GWC has the capacity to immobilise Pb via association with Fe/Mn oxyhydroxides and that SCG may immobilise Pb by association with organic matter. It is hypothesised that Pb, as a chemically “soft” metal, may at least in part bind to “soft” sulfur-containing functional groups on the SCG molecules. These large changes are likely to be due to the majority of Pb in the Tyndrum tailings being held in carbonate forms, and so are easily transformed by environmental processes (Hashimoto *et al.* 2011).

Analysis of the GWC amendment highlighted a high concentration of total (~20,000 mg kg⁻¹) and a low concentration of water-soluble Fe (~16.4 mg kg⁻¹) (Table 6-1), which for the latter was suggested as a reason for decreased Pb leaching upon incorporation (Chapter 6, section 6.6.3). It is possible that intermittent cycling of wet and dry conditions at Tyndrum (Appendix 10) over the 7 month period resulted in fluctuating changes in redox and pH conditions, promoting the dissolution of Fe/Mn oxyhydroxides from the GWC and subsequently enhancing Pb sorption processes (Grybos *et al.* 2007; Sun *et al.* 2007; Loomer *et al.* 2011). If this process occurred repeatedly over time, then this would provide a reasonable explanation for the net increase in Pb concentrations in the reducible phase and a decrease in the weakly associated phase when redox sensitive Fe/Mn oxyhydroxides are being solubilised and re-precipitated.

Several authors have remarked that the periodical flooding of soils can result in a net immobilisation of Pb via the dissolution and subsequent removal of Pb – Fe oxy-hydroxides from solutions (Charlatchka and Cambier 2000b; Chrastný *et al.* 2006; Du Laing *et al.* 2009; Mukwaturi and Lin 2015; Ciszewski and Grygar 2016), however it is important to consider the reversibility of these

reactions, as previously bound Pb can be re-mobilised during periods of flooding (Contin *et al.* 2007; Du Laing *et al.* 2009). Interestingly, the application of GWC indicated a reduction of Pb bound to stable OM and sulphides, proposing that Pb bound to functional groups on OM surfaces may be liberated. Reasons for a decrease in the proportion of Pb present in this fraction may be applicable, such as chemical degradation from plant root exudates, which can release oxidising chemicals into the soil system, and through microbial degradation (Unz and Shuttleworth 1996; Gadd 2000; Dakora and Phillips 2002). Although this fraction represents a minority of the total Pb in the un-amended tailings (~ 12%, ranging between 1,200 – 4,700 mg kg⁻¹) it suggests that, to some extent, an increase in Pb availability may arise from the incorporation of GWC, although it appears that liberated Pb from this fraction was incorporated into the reducible phase.

Regarding SCG, the incorporation of this material demonstrated a significant increase in Pb concentrations in the reducible and oxidisable fraction, with a reduction of 39% in the weakly associated Pb fraction under this treatment group. This shift of Pb into less labile fractions indicates a substantial immobilisation effect of Pb in SCG treated tailings and suggest a lower potential for Pb leaching or mobility in the tailing system. This significant incorporation of Pb into the oxidisable phase is most likely due to the introduction of reactive organic ligands or possibly stable sulphonate groups, which may be promoting the creation of inner-sphere bonds and stable humic-metal complexes, thus reducing the mobility of Pb in the tailing system (Tokimoto *et al.* 2005; Kim *et al.* 2014; Seniūnaitė *et al.* 2014; Wu *et al.* 2016). Furthermore, the increase in reducible Pb may be due to Mn oxy-hydroxide scavenging of Pb as explored in Chapter 6, (section 6.7.5.2.), further reducing the proportion of weakly associated Pb.

The fixation of Pb into the oxidisable and reducible fractions by SCG suggests that the amended tailings would be less likely to release Pb under changing environmental conditions, such as in the acidification and chemical alteration of the rhizosphere and by changes in redox conditions (Fayiga and Saha 2016;

Kabas *et al.* 2017; Boonsrang *et al.* 2018; Lwin *et al.* 2018). However, despite the reductions in weakly associated Pb in the SCG-amended tailings, a significant concentration of Pb remains (3,800 – 12,600 mg kg⁻¹) in this fraction and may continue to present an environmental hazard over time.

Comparisons of the BCR-fractionation of Pb in Chapter 6, (section 6.7.4) and this investigation indicate a substantial transition over time, with evidence of increased fixation of Pb into the reducible and oxidisable fractions in the GWC and SCG treatments respectively. Comparing the two investigations, it could be argued that fixation of Pb by Mn/Fe oxy-hydroxides is a fast reaction, driven by soluble concentrations of Mn or Fe initially, and then potentially by vegetative influences or changes in redox by periodic flooding of the tailings over time. The large concentration of Fe in the GWC amendment may have facilitated this cycling and the greater representation of Pb in this fraction, where contrastingly, more chemically active organic ligands in the SCG may have induced the Pb binding to the oxidisable fraction over time (Kim *et al.* 2014).

Total concentrations of Pb in the roots of sampled vegetation were not influenced by amendment incorporation. However BCs were significantly reduced, demonstrating a decrease in relative uptake after OM introduction as compared to the un-amended control. The reasoning for this can be attributed to the same arguments of site heterogeneity as discussed in section 7.4.6.4. Furthermore, translocation factors of Pb from the root to the shoot were significantly decreased under SCG and GWC amendments, as were total shoot concentrations in vegetation growing in the GWC amended plots. This indicates that the application of amendments incur resistance to the accumulation of Pb into plant tissues, but also suppress the transportation of Pb to aerial compartments, limiting the total concentration of Pb that may be available to herbivores. Similar findings are abundant in the literature concerning reduced Pb uptake and translocation under organic amendment additions (Pardo *et al.* 2017b; Forján *et al.* 2018; Pukalchik *et al.* 2018), and are suggested to be due to decreased bioavailability and improved plant resilience. Despite this, concentrations in the shoots of all sampled vegetation exceeded 1,000 mg kg⁻¹, indicating a significant

accumulation in aerial compartments, and therefore posing a risk to herbivorous activities (Bilal Shakoor *et al.* 2014; Gramss and Voigt 2014), although as indicated previously, this impact is likely to be minimal in the surrounding environment.

Lead uptake by vegetation was strongly correlated to the weakly associated fraction of the BCR procedure, with correlation coefficients of up to 0.98 for total plant Pb concentrations. As Pb is predominately held in carbonate structures at Tyndrum, the action of a weak organic acid as presented as 0.11 mol L⁻¹ acetic acid clearly represents the uptake of Pb by *A.capillaris* as compared to the CaCl₂ extractions which underestimated Pb uptake. It is evident that the first stage of the BCR-protocol mimics plant root exudates at this site, and probably relates well to the mobilising effect of low molecular weight organic acid introduction by plant roots (López-Bucio *et al.* 2000; Shan *et al.* 2003; Feng *et al.* 2005). Furthermore, it gives evidence of the immobilisation effect of GWC and SCG, indicating that the shift of chemical fractions presents a convincing improvement in the reduction of Pb toxicity and uptake by *A.capillaris*. Therefore, the first stage of the BCR procedure would be an excellent indicator of Pb availability to plant uptake in future investigations on the Tyndrum mine tailings and may perhaps be applied widely to mine tailings where Pb is predominantly held by carbonate structures.

7.4.6.3 Zn Fractionation and Plant Uptake

The fractionation of Zn was not significantly impacted by the incorporation of SCG or GWC, although the outputs from the ANOM (Appendix 6) and Figure 7-3 suggest a trend of an increasing proportion of Zn in the reducible fraction of SCG in comparison with the opposite trend for in the un-treated tailings. This suggests that there was a limited transfer of Zn held in weakly associated forms to Zn bound by Fe/Mn (hydr)oxides, although the effect was not sufficiently large to be apparent at a significance level of 0.05. Furthermore, the significant finding of increased total Zn by ANOM, with the contradictory finding of no significant concentrations between the 4-step sum of the BCR products or the separate direct *aqua-regia* extraction indicate a compounding effect of site

variance in the statistical testing of treatments. This is likely due to the intrinsic heterogeneity of the site and the high variance observed between groups and plots.

Conversely, significant differences in BCR-fractions were apparent across all treatments, with the residual fraction holding the largest proportion of Zn and the reducible fraction the lowest. This demonstrates that a large proportion of Zn is generally unavailable for biotic interaction as it is held in stable mineral structures. As discussed in Chapter 6, (section 6.7.5.3), the strong competitive effect of Pb for oxy-hydroxide species combined with a higher overall concentration of Pb in the tailing matrix likely out-competed Zn for binding sites. This possibly limited the immobilisation effect of Zn-Fe/Mn reactions (Covelo *et al.* 2007; Lu and Xu 2009). Under GWC additions, a similar non-significant trend could be drawn from the oxidisable fraction, where a small increase was observed. This may indicate that a limited proportion of Zn was immobilised by stable organic compounds or held in similarly resistant structures.

In contrast to the findings discussed in Chapter 6, there was no evidence of the weathering of the residual phase of Zn and the possible incorporation of Zn into the oxidisable pool, so in the long-term under natural conditions this process may not occur. The increased temperature and lack of vegetation observed in Chapter 6 may have amplified the microbial degradation of mineral phases, a phenomenon that may not translate under more complex environmental conditions (Faimon and Lang 2018; Sun *et al.* 2018; Zou *et al.* 2018). Overall, it can be summarised that the impact of organic amendments on the fractionation of Zn are limited and potentially compounded by site heterogeneity and the high competitive effect of Pb for available binding sites.

Vegetative uptake of Zn was also not significantly different in terms of total tissue Zn concentrations; however GWC was found to increase the relative uptake of Zn into plant systems in comparison with un-amended tailings and SCG. It is possible that a reduction in plant stress elicited an increased uptake in Zn in comparison to vegetation in un-amended plots, however a similar finding may be expected in the SCG treatments. Considering total elemental

concentrations of Zn by plot and treatment (Appendix 11) it appears that half of the replicated plots had higher weakly associated, CaCl_2 – extractable and total Zn concentrations in the GWC treatments, and this is probably the underlying reason for the increased BCs observed. Whilst GWC had $\sim 240 \text{ mg kg}^{-1}$ total Zn (Table 6-1), it is unlikely that the incorporation of this material was the sole factor leading to an increased uptake of this element.

Ecological risks considering the elevated plant uptake of Zn are not considered to be significant in the wider literature, and although a few studies have indicated the use of elevated Zn concentrations in above ground tissue as a deterrent towards herbivores (Kazemi-Dinan *et al.* 2014; Ceacero *et al.* 2015; Stolpe *et al.* 2017), incidents of Zn poisoning are not believed to be associated with the consumption of Zn enriched plant material as discussed in Chapter 2, (section 2.5.2) (ATSDR 2005; Plum *et al.* 2010) . However, elevated concentrations of Zn in plant tissues shoots may well be contributing to biotic stress and the reduction of vegetative growth (García-Gómez *et al.* 2018; Paunov *et al.* 2018).

In parallel to the prediction of Pb availability, Zn uptake into plant tissues was suitably explained by the weakly associated fraction of the BCR procedure ($R^2 = 0.91$), indicating the usefulness of this extraction for predicting Zn concentrations at the Tyndrum mine. Furthermore, this suggests that if a decrease in weakly associated Zn can be achieved then it is likely that a decrease in plant uptake of Zn will occur.

7.4.6.4 Site Heterogeneity and Implications for Revegetation

The field trials suggested site heterogeneity to be a dominant factor in terms of total, extractable, and plant available PTEs, as well as pH and particle size. The variation in these parameters was substantial, as can be seen in the total concentrations of PTE between plots approximately 30 cm apart (Appendix 11). This variable and unpredictable disparity in tailing characteristics presents an issue when attempting to generalise remedial methods across a single site, and an intractable problem if approaches are intended to be applied to dissimilar mine tailings. For example, the high concentrations of PTE in the tailings from plot 5 (Figure 7-4), greatly inhibited plant development across all treatments,

and the small median particle size ($\sim 100 \mu\text{m}$) may have also inhibited plant development due to compaction.

Many studies cite the usefulness of phytoremediation as demonstrated in field trials. However, trials are often confined to a single area of the mine tailing, and so do not capture the effectiveness of the remedial approach across such diverse landscapes. Therefore, unless field trials encompass the full variation of the mine site, or at least attempt to include obvious environmental gradients, the confidence in the remedial effectiveness will be diminished. Whilst this study has been constrained in terms of physical plot size, ($\sim 0.13 \text{ m}^2$), it has captured the variability across the site, and therefore a greater confidence in the general effectiveness of the approach can be drawn. Phytoremediation at Tyndrum would likely be highly successful in the areas where weakly associated concentrations of PTE are lower and particle size is higher ($>500 \mu\text{m}$), which tentatively could be found in the central area of the site based on the findings in Chapter 1. Contrastingly, sections of the mine near the adjacent waterways would provide difficulties in vegetative development, owing to the likelihood of diminished plant growth due to flood stress and possibly elevated PTE.

7.4.5 Conclusion of Section

Field trial findings suggest that the incorporation of GWC and SCG generally double vegetative growth in the former and enhance growth by approximately five times in the latter. Furthermore, both amendments suppressed the uptake of Cd and Pb, limiting the phytotoxic effects of these elements to *A. capillaris* and improving vegetative establishment. Moreover, the study has highlighted the usefulness of the BCR weakly associated fraction for predicting plant uptake of Pb and Zn, allowing this to be a useful metric for future investigations. Issues associated with site variability on the effectiveness of the remedial approaches highlighted the importance of ensuring that phytoremediation methodologies at the site encompass the full range of tailing characteristics. Finally, it would be recommended that any further field trials would be completed on a larger scale (10 m^2) using SCG or organic amendments with similar chemical and physical characteristics as outlined in Table 6-1.

7.5 Le Bleymard Field Trial

7.5.1 Calcium Chloride – Extractable Cd, Pb and Zn concentrations

Calcium chloride extractable Cd, Pb and Zn concentrations were not significantly different across treatments or plots in the exception of Pb in the GWC group, which had significantly lower CaCl₂-extractable Pb than the un-amended control (Figure 7-13). Concentrations of CaCl₂-extractable PTE were low and did not represent more than 0.05% of total concentrations across all elements and treatments.

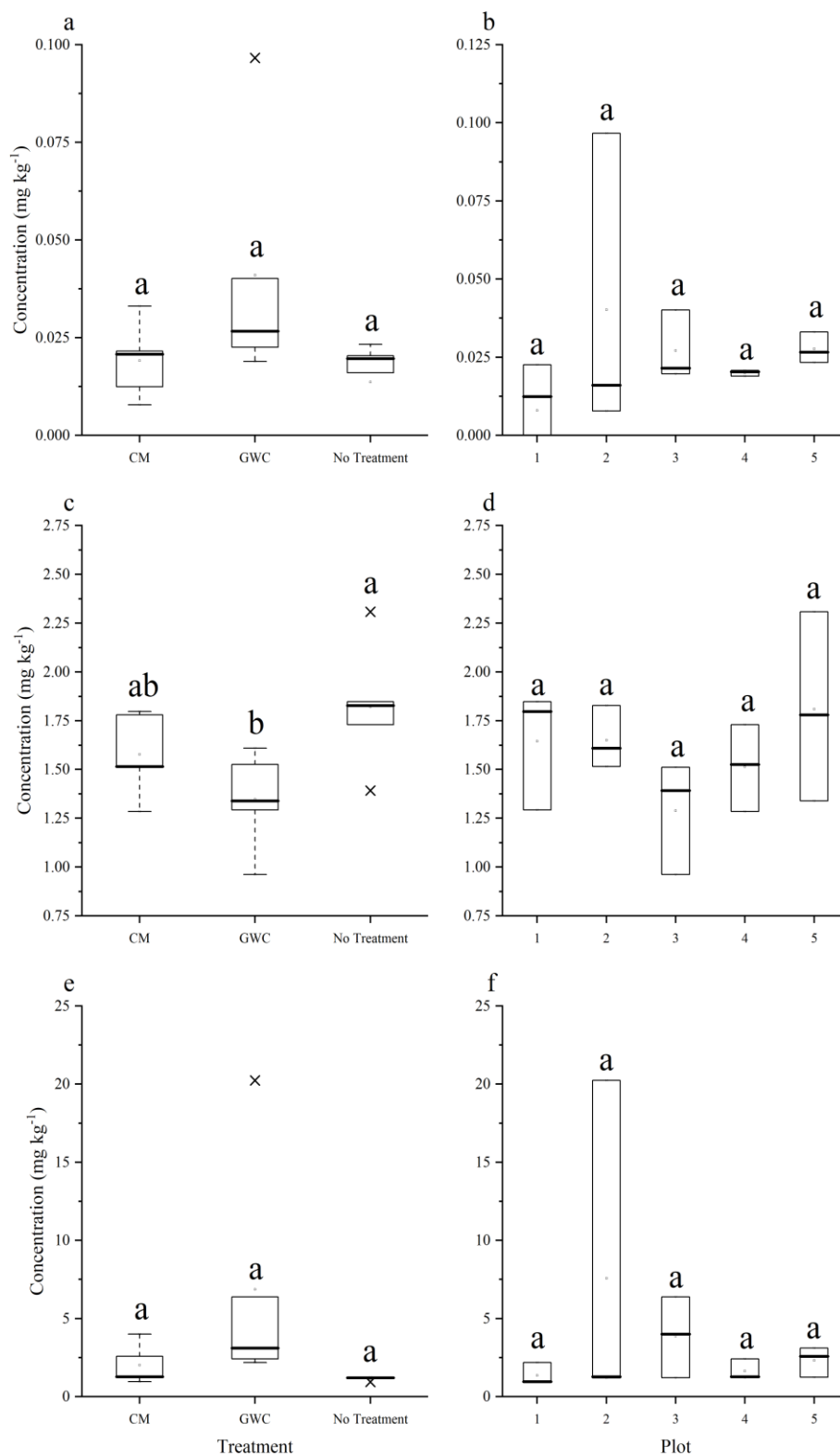


Figure 7-13. Calcium chloride - extractable Cd (a–b), Pb (c–d) and Zn (e–f) concentrations from the Le Bleybard field site by treatment (LH) and by plot location (RH). Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$ n = 5).

7.5.2 EDTA – Extractable Cd, Pb and Zn

The concentrations of EDTA extractable – Cd and Pb were not significantly affected by treatment groups, however, EDTA extractable Zn was significantly reduced in the tailings treated with GWC (Figure 7-14). Concentrations of EDTA extractable – Cd ranged between 35-82 mg kg⁻¹, 740 – 2,400 mg kg⁻¹ for Pb and 4,600 – 7,000 mg kg⁻¹ for Zn, equating to approximately 32.9 (± 6.7), 25.5 (± 6.7) and 19.6 (± 3.0) percent of the total concentration for each element respectively.

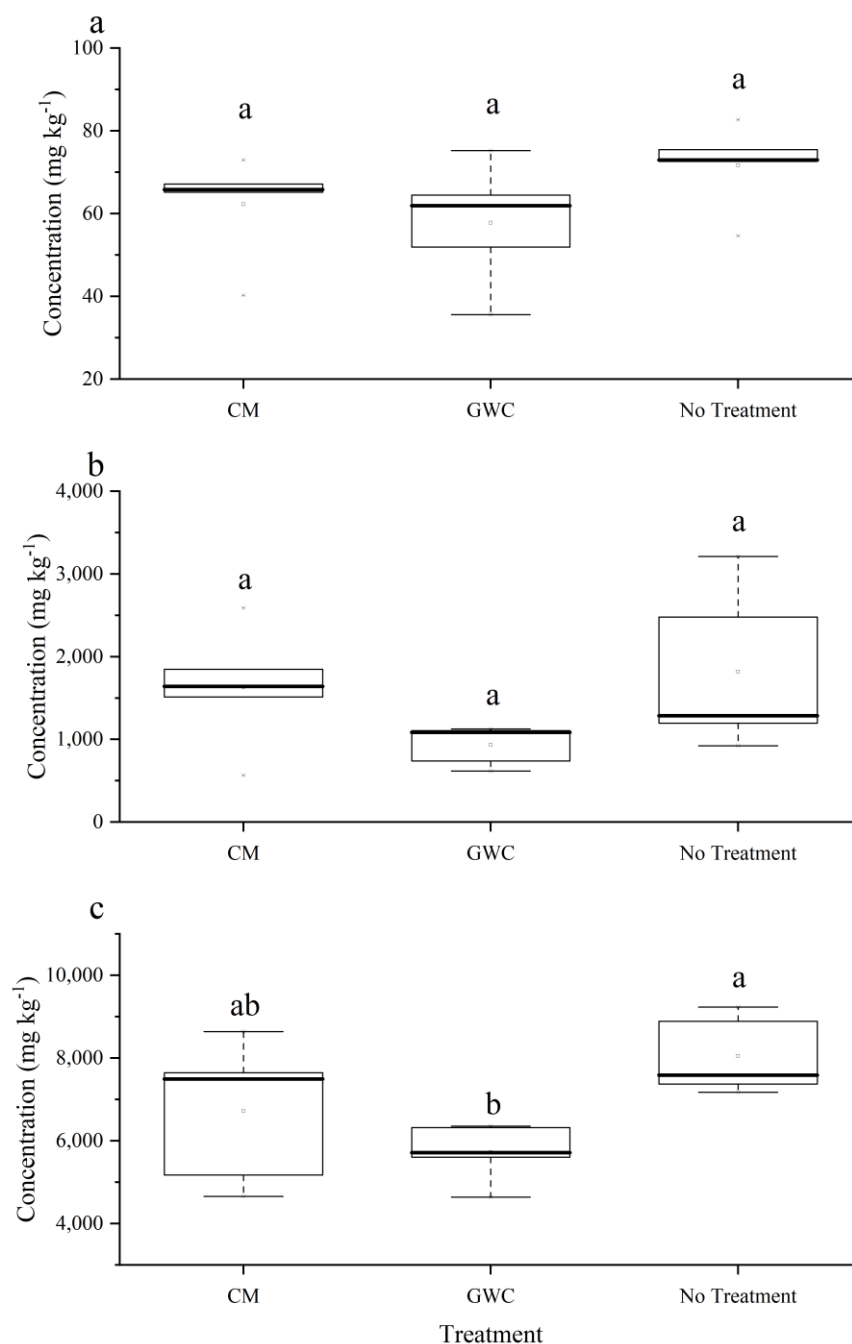


Figure 7-14. Concentrations of EDTA-extractable Cd (a), Pb (b) and Zn (c) from the Le Bleymard site. Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$ n = 5).

7.5.3 Plant Growth, Cover and PTE Uptake

7.5.3.1 Vegetation Height and Biomass

Vegetation height was significantly higher in the GWC treatments (Figure 7-15) as compared to tailings amended with CM or the untreated tailings, however

percentage cover was not. *A.capillaris* was visually identified as the dominant species, with only a few occurrences of *F.ovina* reported across all treatments.

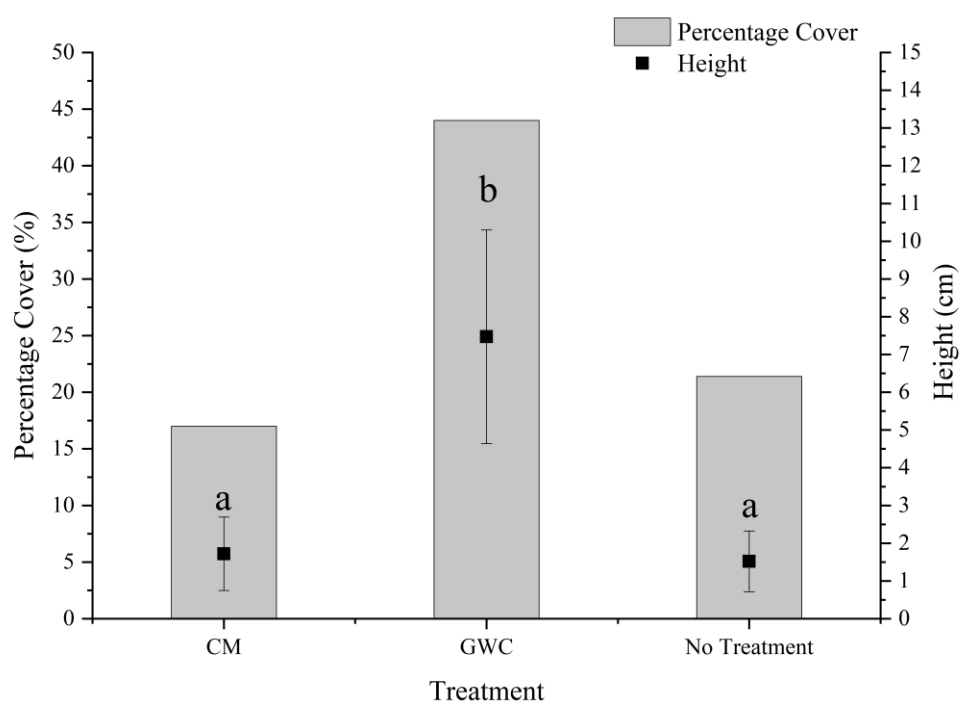


Figure 7-15. Vegetation height and percentage cover of treated and un-amended tailings at the Le Bleymard mine. Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$ n = 5).

Shoot and root biomasses were significantly higher following the GWC treatment as compared to the CM amended tailings and the un-treated control (Figure 7-16). No significant differences in root or shoot biomass were observed between plots (data not shown, $p = > 0.05$).

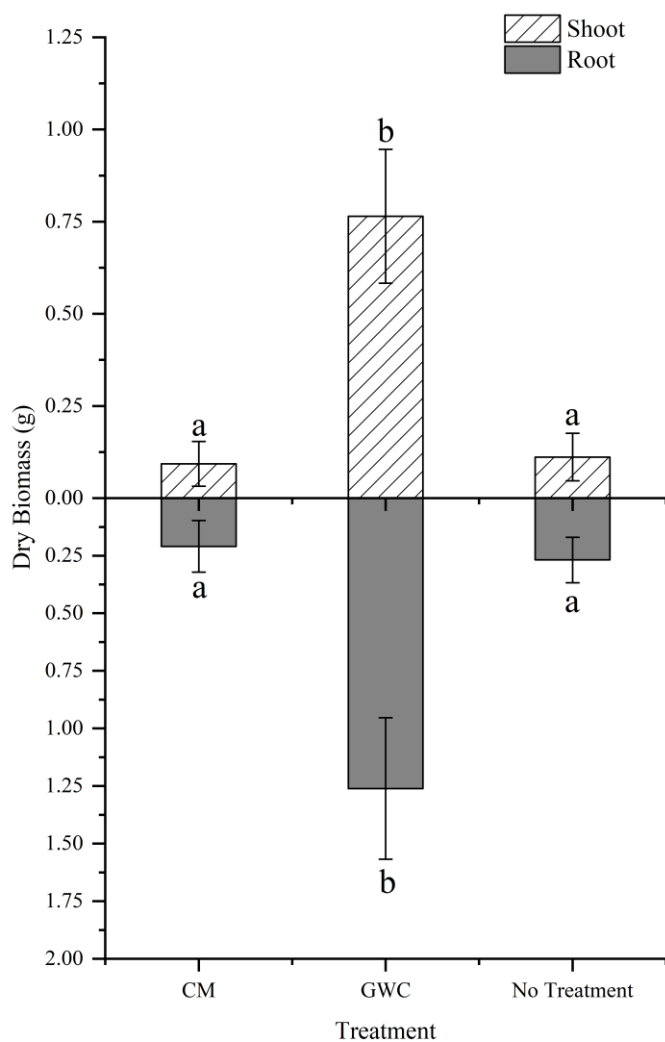


Figure 7-16. Shoot and root biomass of treated and un-amended tailings at the Le Bleymard site. Means that do not share a letter are significantly different for aerial and below ground components respectively ($\alpha = 0.05$).

7.5.4 PTE Concentrations in the Shoots and Roots of Plants.

7.5.4.1 Cd

Cadmium concentrations were significantly higher in the shoots of the untreated tailing vegetation, with the applications of CM and GWC significantly decreasing Cd concentrations in shoot biomass in that order (Figure 7-17). Root uptake of Cd was significantly higher in the un-amended tailings as compared with the CM and GWC treatments, with no significant differences observed between treatment groups. Overall, plant uptake of Cd was significant across all treatments, ranging from 22 mg kg⁻¹ in the GWC treatments to 162 mg kg⁻¹ in the un-treated tailings.

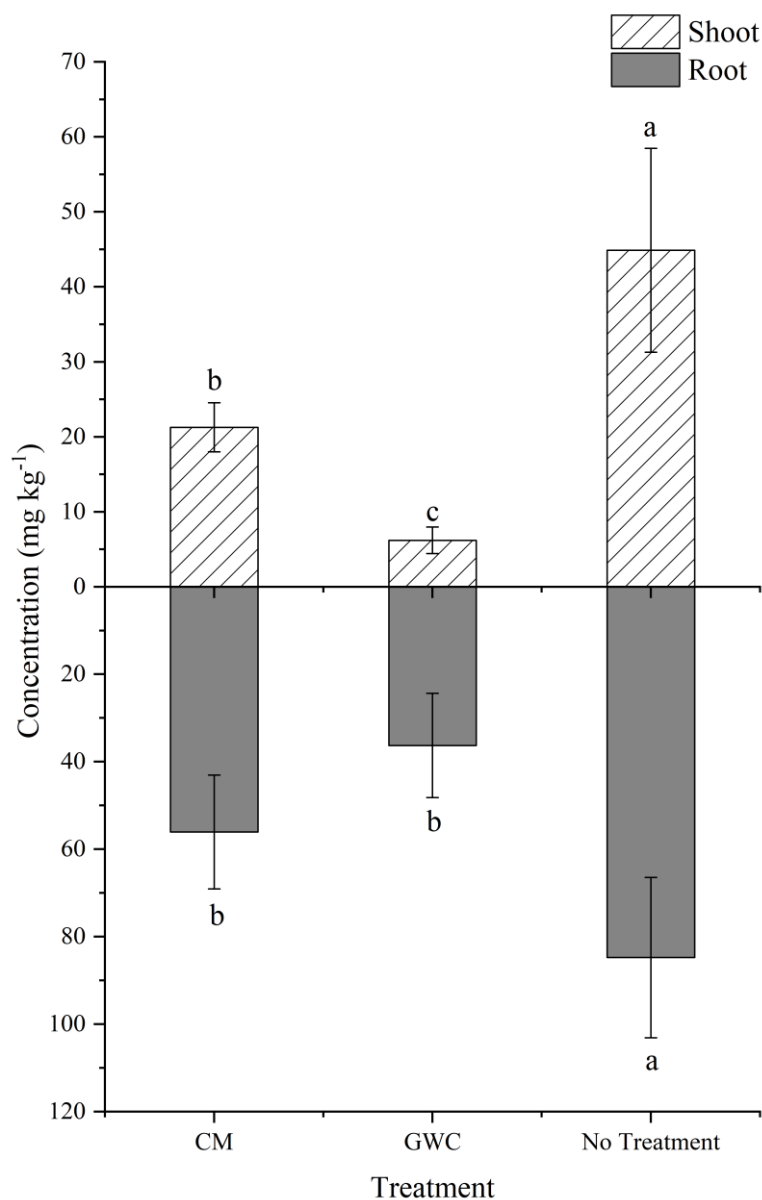


Figure 7-17. Cadmium concentrations in the roots and shoots of the untreated and amended tailings at the Le Bleymard site. Means that do not share a letter are significantly different for aerial and below ground components respectively ($\alpha = 0.05$, $n = 5$).

7.5.4.2 Pb

Shoot concentrations of Pb were significantly higher in the plants growing in the un-treated tailings as compared to CM and GWC-amended tailings (Figure 7-18). No significant differences in the root uptake of Pb was observed between treatment groups. The total mean concentrations of Pb in plant tissues were 1,370 (± 732), 1,094 (± 347) and 2,567 ($\pm 1,494$) mg kg⁻¹ for CM, GWC and the un-treated control respectively.

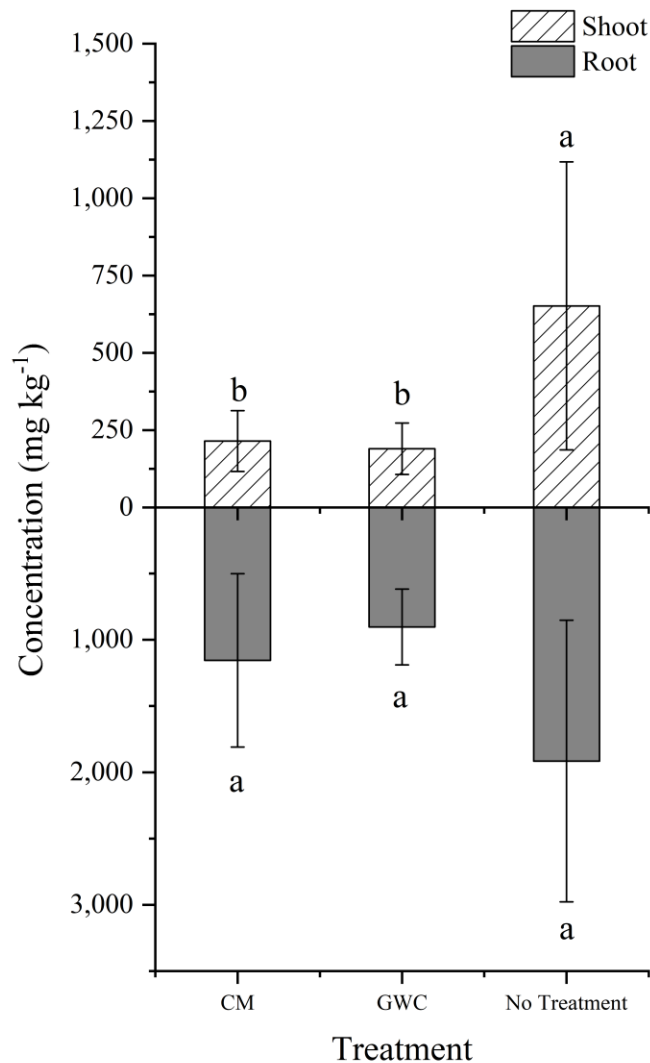


Figure 7-18. Lead concentrations in the roots and shoots of the untreated and amended tailings at the Le Bleyard site. Means that do not share a letter are significantly different for aerial and below ground components respectively ($\alpha = 0.05$, $n = 5$).

7.5.4.3 Zn

The concentrations of Zn in the shoots of sampled vegetation were significantly lower in the GWC treatments as compared to the CM and un-treated tailings (Figure 7-19). Furthermore, the tailings treated with GWC and CM also had a decrease in root Zn uptake in comparison with the un-amended tailings. Mean total concentrations for Zn in plant biomass were 7,200 ($\pm 2,300$), 3,800 (± 889) and 10,000 ($\pm 2,200$) mg kg⁻¹ for CM, GWC and the un-treated controls respectively, with the GWC treatments demonstrating a significantly lower Zn uptake in comparison to both other treatments.

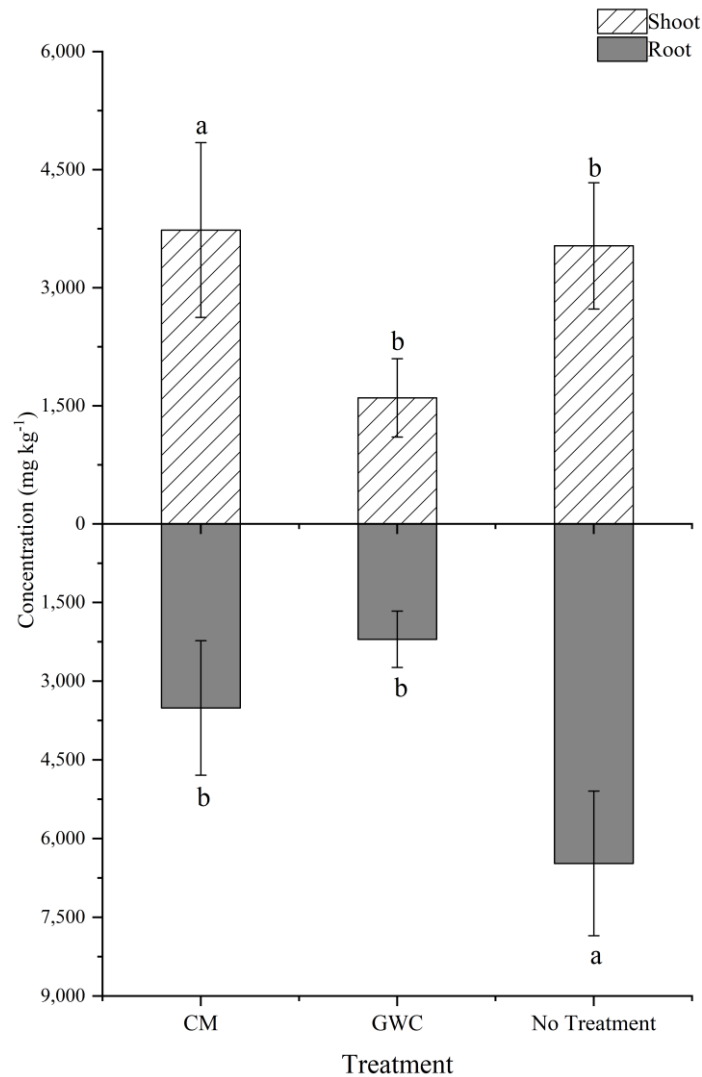


Figure 7-19. Zinc concentrations in the roots and shoots of the untreated and amended tailings at the Le Bleymard site. Means that do not share a letter are significantly different for aerial and below ground components respectively ($\alpha = 0.05$, $n = 5$).

7.5.4.4 Translocation and Bioaccumulation Factors

The mean BCs and TFs for the treatment groups and related elements are described in Table 7-5. The BC for Cd were significantly lower for vegetation growing in tailings amended with CM and GWC, with the GWC applications also decreasing the TF for Cd. Similarly, Pb BC was decreased in the treated tailings, with CM suppressing Pb translocation to the shoots in comparison to the GWC and un-treated groups. Zinc BC were lowest in the GWC group, whereas CM did not exhibit significantly lower BC as compared to the un-amended control. The TF for Zn was significantly increased in the CM treatment,

with a greater translocation of Zn in the shoots in comparison to the roots, however Zn TF did not differ between GWC and un-treated controls.

Table 7-5. Bioaccumulation coefficients (BC) and translocation factors (TF) for Cd, Pb and Zn in sampled vegetation by treatment at the Tyndrum mine tailings. Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$ n = 5) and are highlighted in bold.

Element	Treatment	BC	TF
Cd	CM	0.39 (± 0.05) ^a	0.39 (± 0.08) ^a
Cd	GWC	0.27 (± 0.03) ^a	0.17 (± 0.02)^b
Cd	No Treatment	0.53 (± 0.11)^b	0.52 (± 0.11) ^a
Pb	CM	0.21 (± 0.05) ^a	0.20 (± 0.06)^a
Pb	GWC	0.24 (± 0.01) ^a	0.21 (± 0.07) ^{ab}
Pb	No Treatment	0.41 (± 0.06)^b	0.33 (± 0.09) ^b
Zn	CM	0.21 (± 0.07) ^{ab}	1.14 (± 0.3)^b
Zn	GWC	0.13 (± 0.03)^b	0.74 (± 0.19) ^a
Zn	No Treatment	0.26 (± 0.06) ^a	0.54 (± 0.04) ^a

7.5.5 Nitrogen, Carbon, Water soluble P Concentrations and pH

7.5.5.1 Total Nitrogen and NO₃⁻/ NH₄⁺ -N Concentrations

Total N was observed to be significantly higher in the tailings treated with GWC, whereas CM treated material was not (Table 7-6). Concentrations of extractable NO₃⁻ and NH₄⁺ were highly variable and did not differ significantly between treatment groups or plot locations.

Table 7-6. Measured N parameters in the treated and untreated mine tailings at Le Bleymard. Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$). n = 5 and are highlighted in bold.

Treatment	Total N (%)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)
CM	0.03 (± 0.02) ^{ab}	1.94 (± 1.9) ^a	2.57 (± 2.6) ^a
GWC	0.06 (± 0.03)^b	16.88 (± 17.0) ^a	5.53 (± 3.3) ^a
No Treatment	0.02 (± 0.01) ^a	4.86 (± 3.0) ^a	4.4 (± 6.6) ^a

7.5.5.2 Total Carbon and Hot and Cold Water Extractions

Total C was significantly higher in the GWC treated tailings in comparison to the CM and un-treated controls (Table 7-7). Cattle manure did not influence TC in comparison to the GWC amended controls. No statistical differences were

observed in the CWEC fraction between treatment groups, however GWC was found to have significantly higher concentrations of HWEC in comparison to the un-amended controls.

Table 7-7. Measured N parameters in the treated and untreated mine tailings at Le Bleymard. CWEC = Cold water extractable carbon, HWEC = Hot water extractable carbon. Means that do not share the same letter are significantly different at a value of ($\alpha = 0.05$). n = 5 and are highlighted in bold.

Treatment	Total OC (%)	CWEC (mg kg ⁻¹)	HWEC (mg kg ⁻¹)
CM	8.63 (± 1.7) ^a	119 (± 32.6) ^a	310 (± 85.0) ^{ab}
GWC	10.6 (± 0.88)^b	102 (± 13.1) ^a	367 (± 178)^b
No Treatment	8.38 (± 1.7) ^a	102 (± 24.1) ^a	145 (± 32.9) ^a

7.5.5.3 Water Extractable P

The concentrations of water-extractable P were not significantly different between CM (3.47 ± 2.74 mg kg⁻¹), GWC (3.32 ± 1.68 mg kg⁻¹) and un-treated controls (1.74 ± 0.71 mg kg⁻¹) ($p = > 0.05$).

7.5.5.4 Tailing pH

The pH of the tailings were not significantly different between treatment groups and the un-amended control ($p = > 0.05$) with an overall geometric mean of 7.5 (± 0.3). In general there was no significant difference in the pH across plot locations, in the exception of plot 4, which had a geometric mean of 8.1 in comparison to the average value of 7.5.

7.5.6 Prediction of PTE Bioavailability

Concentrations of Cd, Zn and Pb extracted by 0.01 M CaCl₂ did not correlate strongly with plant tissue concentrations for these PTEs (Figure 7-20). Concentrations of EDTA-extractable PTE were strongly correlated with Pb concentrations in roots ($R^2 = 0.85$), total plant concentrations ($R^2 = 0.9$), and moderately correlated for Zn concentrations in the root system ($R^2 = 0.6$), (Appendix 7). Cadmium uptake and concentrations in the shoots or roots of the plants were underestimated by Cd concentrations following CaCl₂ and EDTA extractions.

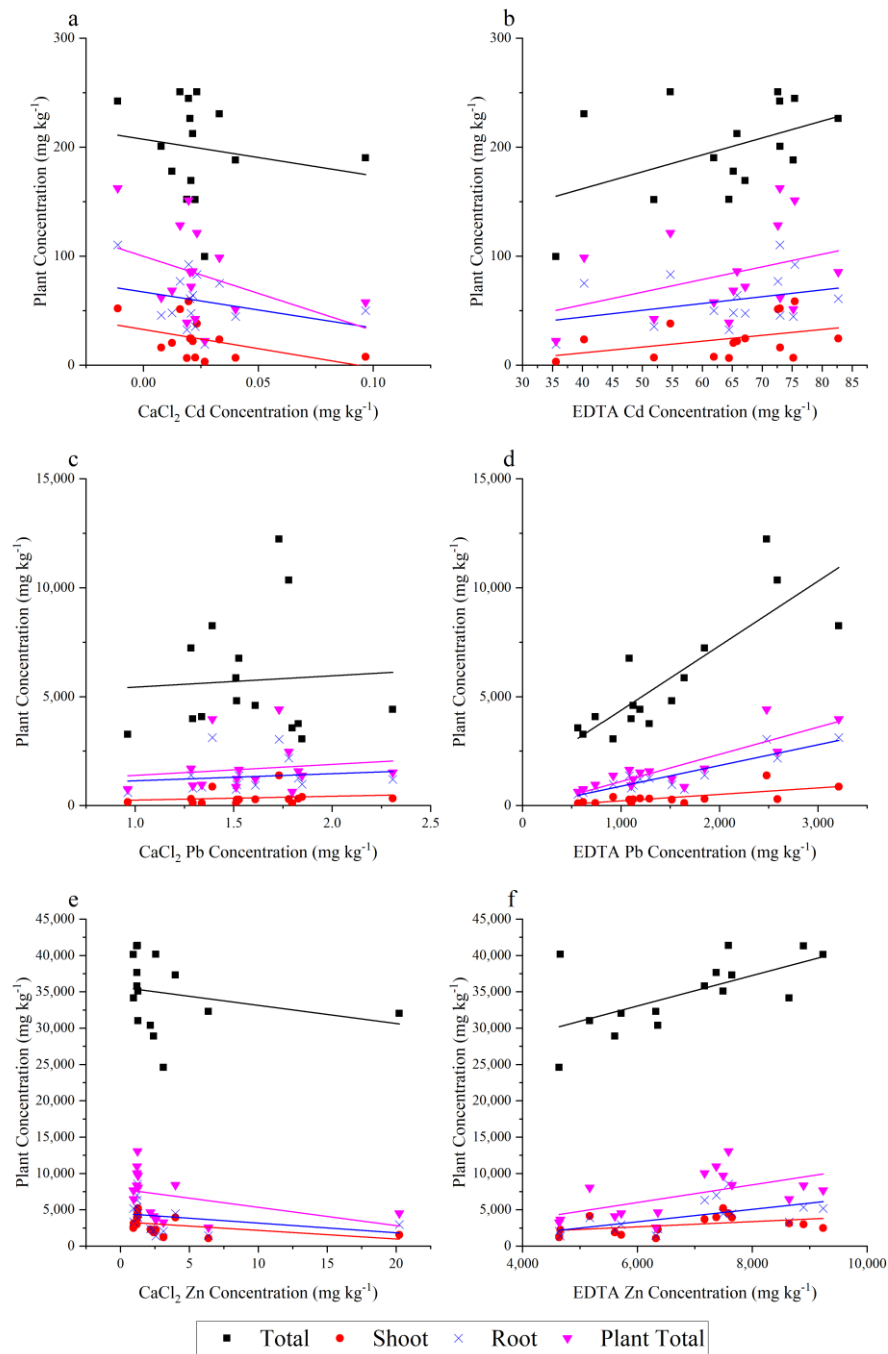


Figure 7-20. Correlations between plant PTE concentrations, total tailing concentrations and CaCl₂/EDTA extractable Cd (a-b), Pb (c-d) and Zn (e-f) at the Le Bleymard site.

7.5.7 Discussion of Le Bleymard Findings

7.5.7.1 Vegetation Establishment and Tailing Nutrient Content

Shoot height and root biomass was highest in the GWC group, demonstrating that this amendment enhanced the development and growth of *A. capillaris* in the

Bleybarn tailings in comparison to the CM applications (Figure 7-16). However, the mean percentage cover for GWC across plots ranged between 5 - 85%, indicating that vegetative cover was not consistent across the treatment. Furthermore, shoot and root biomasses were not significantly different between the CM and un-amended tailings, with only an improvement observed in the GWC amended treatments, which had demonstrably greater root biomass in particular.

It is apparent that despite the incorporation of OM via the organic amendments, the development of vegetation in the trial plots was limited. Considering the concentrations of available N, CWEC, and water-soluble P, the amended tailing plots did not exhibit significantly greater available nutrient contents compared with the un-amended controls, although small increases in TN and HWEC were observed in the GWC treatment. The lack of available plant nutrients is likely a significant factor in the poor development of vegetation, alongside the PTE burden that is described in the following sections. From previous investigations (Chapter 6, section 6.4) it is clear that significant leaching of NO_3^- and mineralisation of NH_4^+ can occur in plots amended with GWC and CM, processes that may have resulted in the similarly low N concentrations after the 7-month period, despite amending with a nutrient source. Furthermore, the elevated pH and high concentrations of free Ca^{2+} and Mg^{2+} are likely to have induced the precipitation and immobilisation of several macro and micro plant nutrients, such as Fe, K, Mn, P and Cu (Laliberté *et al.* 2013) by increasing sorption onto solid surfaces and reducing the availability to plants. Several authors have found similar issues regarding the development of vegetation on calcareous soils and mine wastes, with general findings of high P toxicity and low N retention from leaching of NO_3^- from the tailing system (Lambers *et al.* 2008; Li *et al.* 2015; Santini and Banning 2016). Furthermore, the arid climate and dense, small grained physical properties of the tailings will result in a reduced root penetration, water percolation and soil processes, hindering the establishment of vegetation, even when *A. capillaris* has been recognised as a resilient species to hostile soil conditions (Pichtel and Salt 1998; Neagoe *et al.* 2014; Perkins *et al.* 2016). Despite the improvements in vegetative cover under

the GWC treatment it is uncertain that vegetation would be able to sustain several years of growth due to the poor nutrient status and saline stress that may be occurring (Cross and Lambers 2017).

Similarly to the findings at Tyndrum the absence of *F.ovina* suggests that this species was substantially impacted by the conditions encountered in the Le Bleymard tailings. As suggested in section 7.4.6.1 the concentrations of PTE in root and shoot tissues were significantly greater than those encountered in Chapter 5 and were likely a controlling issue in the establishment of *F.ovina*, as the species has previously been observed to populate arid calcareous soils (Otsus and Zobel 2004; Rosén and Bakker 2005). *F.ovina* therefore would not appear to be a suitable phytostabilisation candidate for Le Bleymard, despite evidence for the species tolerating PTE contaminated and calcareous soil systems in the wider literature.

It would appear that higher application rates of GWC would be required to improve the establishment of vegetation, as larger inputs of amendments would arguably increase nutrient content and improve soil structure. However, a recent review of vegetation succession and reclamation of alkaline tailings suggested that the incorporation of organic amendments can be unsuitable for alkaline tailings, primarily due to the issues of N and P loss and the immobilisation of OM and other plant nutrients (Cross and Lambers 2017). The authors suggest that these issues can be circumvented by the use of acidifying media to lower the pH and enable greater mobility of plant nutrients, however this poses the risk of increasing PTE mobility, which is discussed in more detail in further sections.

7.5.7.2 Cd Availability and Plant Uptake

Cadmium uptake into the roots of *A.capillaris* was significantly reduced by the application of cattle and GWC amendments, with a resulting decrease observed in the aerial compartments of the plants (Figure 7-17). Furthermore, BC and, in the case of GWC, TFs were decreased upon incorporation of both amendments. This demonstrates that the application of OM to the Bleymard tailings was effective in decreasing Cd plant uptake and limiting Cd-toxicity to vegetation. Other investigations, where Cd uptake has been reduced by the application of

manure and GWC, have suggested a range of potential mechanisms, such as increased pH and enhanced sorption onto soil surfaces (Ahmad *et al.* 2015; Dahlin *et al.* 2016; Rizwan *et al.* 2016; Khan *et al.* 2018).

Considering that Cd mobility is widely controlled by pH in soil systems, it is likely that the high baseline pH of the Le Bleymard tailings buffers Cd mobilisation, as described in Chapters 4 and 6, possibly by precipitation as Cd(OH)₂. This may have been countered by the presence of root exudates and the acidification of the rhizosphere by plant derived organic acids (Hu *et al.* 2011; Li, Liang, *et al.* 2013; Li, Wang, *et al.* 2014; Wang *et al.* 2017; Mahmud *et al.* 2018), leading to the uptake of Cd observed in all treatment groups. The reduction of Cd uptake observed in the amended tailings may have then been caused by the presence of stable humic compounds introduced by CM and GWC treatments (Hanc *et al.* 2009; Sato *et al.* 2010; Sorrenti and Toselli 2016; Khan *et al.* 2018), effectively limiting the uptake of Cd by reacting and forming stable organic compounds via the reaction with carboxyl and hydroxyl functional groups, (Mahmood 2010). In this investigation plant tissue concentrations of Cd were not correlated with other measured variables, such as pH, TC or extractable C, suggesting that increases in OM, C or changes in pH cannot directly explain the decrease in Cd uptake by *A.capillaris*. However, the increased concentration of HWE and TC in the GWC treatments could be attributed to an increase in stable organic compounds (Landgraf *et al.* 2006; Bu *et al.* 2010; Balaria and Johnson 2013), which may have enhanced Cd immobilisation in the tailings and reduced the translocation to shoots.

Cadmium uptake by plants was underestimated by both CaCl₂ and EDTA extractions. As CaCl₂ and EDTA are intended to mimic exchangeable fractions and the process of chelation in the soil system respectively (Haynes and Swift 1983; Bakircioglu *et al.* 2011), it could be assumed that Cd uptake by plants is not significantly affected in the tailing environment under these broad definitions. Additionally, neither of these extractions indicated a change in Cd concentration when organic amendments were applied to the tailings, implying that Cd behaviour was not measurably altered when using these assessment

approaches and that neither method is useful in the prediction of plant uptake of Cd. Also, these assessment approaches do not enable identification of the potential mechanisms of uptake (e.g. a strong correlation of Cd in roots and CaCl_2 may have indicated that Cd exists as an exchangeable ion in the tailing system). Interestingly, Cd uptake into plant tissues was well predicted by total tailing concentrations, a finding that has been in part supported by McBride, (2002) whereas in the recent and wider literature, Cd availability to plants is known to be highly dependent on soil pH, organic constituents and underlying geochemical characteristics (Merckx *et al.* 2001; Khan *et al.* 2018; Rosenfeld *et al.* 2018; Yang *et al.* 2018). Considering the accepted pH – related pathway of Cd uptake, the previously mentioned plant induced acidification of the rhizosphere may be a significant factor in the mobilisation and uptake of Cd from the Le Bleymard tailings. This gives greater explanation for the lack of correlation to neutral salt CaCl_2 and EDTA extractions to Cd uptake, and could suggest that the majority of the tailing Cd is held in weakly associated carbonate fractions, liable to dissolution by weak organic acids from root exudates, a finding that could be linked to Appendix 1 and the release of Cd under decreasing pH regimes.

In this study rhizosphere tailing material was not specifically collected for analysis and therefore subtle changes in pH may have been masked by bulk soil pH determination. Unfortunately, the application of the BCR or other sequential extraction procedure was not considered to be appropriate due to the reasons outlined in Chapter 6, and so the chemical fractionation of Cd in the Bleymard tailings was not possible. This may have given greater insight into Cd uptake pathways or the possible mechanisms of Cd immobilisation by GWC and CM.

It would appear that the application of GWC to the tailings at Le Bleymard provide an excellent substrate for lowering Cd uptake and translocation, although conclusive identification of the mechanisms behind the amelioration elude the current investigation, again supporting the findings of (Kumpiene *et al.* 2017) who indicated that Cd availability was highly site-specific. It does appear, however, that the elevated pH of the tailing substrate does not confer

protection to plants in terms of Cd uptake alone, as rhizosphere acidification may be opposing the buffering capacity of the tailing matrix in this regard. Moreover, the addition of OM and stable organic compounds may play a critical role in the impedance of Cd uptake by *A.capillaris* on the Le Bleymard tailings. Finally, despite significant decreases of Cd uptake under organic amendment applications, it is important to highlight that concentrations of $6.2 (\pm 1.8) \text{ mg kg}^{-1}$ of Cd remain in the aerial tissues. This could pose a significant hazard to grazing herbivores, with insects particularly vulnerable to Cd toxicity (Scheirs *et al.* 2006; Clemens and Caroline 2016).

7.5.7.3 Pb Availability and Plant Uptake

The application of GWC resulted in a reduction of CaCl_2 extractable Pb in the tailing system and a strong correlation between EDTA-extractable Pb and total plant accumulation. The application of organic amendments did not reduce the total concentrations of Pb in the roots across treatments, however a decrease in the BC was observed for both CM and GWC amendments, indicating that the use of CM and GWC suppressed Pb uptake, and in relation to CM, the translocation of Pb from roots to shoots. As found previously in *section 7.4.6.3*, conflicting findings of reduced BC and TF with reported tissue concentrations were largely due to differences between Pb concentrations between plots and treatments, and so the use of plant uptake metrics is likely to be more useful than actual concentrations of plant tissue PTE in heterogeneous sites. Therefore, it can be stated that the addition of GWC and CM decreased Pb toxicity and uptake to *A.capillaris*, although the extent of this effect may be inconsistent.

The decrease of Pb uptake may be attributable to the reaction of Pb with OM and the formation of stable organic complexes, a finding that is well reported in the wider literature as the affinity for Pb and organic compounds has been clearly described for GWC and CM (Hashimoto *et al.* 2011; Kamari *et al.* 2015; Staugaitis *et al.* 2016; Chu *et al.* 2017; Lago-Vila *et al.* 2017a; González-Valdez *et al.* 2018). Also, as suggested previously the reasons for the decrease may include conflicting pH/rhizosphere interactions, with the reduction of Pb root accumulation facilitated by the formation of stable organic–Pb complexes due

to an increased presence in organic compounds in the tailings (Li, Tao, *et al.* 2013; Mousavi *et al.* 2018; Zhan *et al.* 2018). Indeed, the BC for Pb was decreased by 50% upon incorporation of GWC and CM, although the exact mechanisms are not clear.

The use of EDTA as a predictor for *A.capillaris* uptake of Pb from the tailings was found to be effective, explaining 90% of the total variation in plant uptake of Pb. This would therefore be an excellent assay for future studies if the prediction of Pb uptake by *A.capillaris* and possibly other species was undertaken. Considering the chemical nature of EDTA extractions as a chelating agent, the complexation and resulting solubilisation of Pb using this technique indicates that *A.capillaris* can liberate Pb weakly held to soil surfaces and a range of other chemical fractions, including perceivably stable organic complexes (Huang *et al.* 1997; Najeeb *et al.* 2017; Qiao *et al.* 2017). This suggests that although some decrease in phytoavailable Pb is assumed when introducing OM, only the most stable complexes will be unavailable for plant absorption. It would therefore be important to attempt to understand the fractionation and association of Pb within the tailing system at Le Bleymard to attempt to identify which amendments can provide highly stable binding mechanisms and relate this directly to plant uptake of Pb.

The findings of this investigation agree with those in Chapter 6, where a reduction in 'bio-available' Pb was assumed upon the addition of CM and GWC. Nevertheless, it is clear that a significant concentration of Pb is absorbed by *A.capillaris* and translocated into the shoots of the grass, despite the reductions granted by the addition of amendments. However, these concentrations would not likely be significant to severely impact herbivorous activities (Gramss and Voigt 2014; Aslam *et al.* 2015).

Overall, it appears that the application of CM and GWC is effective in decreasing Pb uptake by *A.capillaris* at the le Bleymard tailings, and can to some extent reduce the translocation of Pb from roots to shoots. However, it is suggested here that the use of GWC is preferred, due to the increase in plant biomass observed in this treatment group (Figure 7-16).

7.5.7.4 Zn Availability and Plant Uptake

Zinc concentrations as determined by CaCl_2 extractions were not statistically different across treatments or plots, in contrast to EDTA extractable Zn where significantly lower concentrations were identified in the GWC treatments. The uptake of Zn into plant roots across all treatments was higher than Pb and Cd uptake, most likely due to the exceptionally high total concentrations encountered at the tailings in Le Bleymard (Chapter 4, *section 4.6.1*). The application of CM and GWC decreased root concentrations of Zn by 46 and 66% respectively in comparison to the control, indicating that the input of both amendments can be useful in controlling Zn adsorption in the tailings. Furthermore, GWC incorporation decreased shoot tissue concentrations of Zn, suggesting that the translocation of Zn to aerial biomass may be mitigated using this organic material. The wider generalisation of these materials in their capacity to sequester Zn is debatable, and is likely to depend on the source material and composing approach (Kissinger *et al.* 2007; Muscolo *et al.* 2018), however there is good evidence of their successful application for Zn alleviation in the Le Bleymard tailings.

Assessing BC and TF metrics alongside total uptake suggest that the use of GWC is generally more effective in minimising excessive uptake of Zn, whereas no differences were observed between CM and the un-amended tailings. Interestingly, the translocation of Zn was significantly increased upon the application of CM, with a TF of over 1, proposing that *A.capillaris* displays phytoextractive properties of Zn under this organic material (van der Ent *et al.* 2013). Bioaccumulation factors for Zn were generally low in comparison to Cd and Pb, probably due to the significant enrichment of Zn in the tailing substrate and a maximum threshold for Zn accumulation. However, TF values were mostly higher than for Pb and Cd, indicating a preference for the translocation of Zn into the aerial compartments of the plant. This has been widely demonstrated as a common feature in soil conditions of elevated Zn concentrations (Broadley *et al.* 2007) and possibly due to the requirement of Zn within plant proteins in shoot tissues (Haydon 2014). It has been demonstrated that plant uptake of Zn is elevated across all treatments, although uptake and

translocation could be mitigated or enhanced using GWC or CM respectively. Considering the aim of phytostabilisation it would be considered that GWC would be the preferred material, however, CM could be a viable alternative for phytoextraction purposes.

Similarly to Cd and Pb, CaCl_2 and EDTA extractions did not provide strong evidence for predicting plant availability or estimating potential reductions in bioavailability in the amended or un-treated tailings, although lower concentrations of EDTA extractable Zn did mirror a decrease in plant uptake in the GWC group. The applications of EDTA as a predictive tool for plant Zn uptake therefore could provide a conservative estimate of Zn availability to *A. capillaris* across the site, although inferences must be treated with caution.

Similarly to Cd and Pb, it is difficult to describe the mechanisms behind the decrease in Zn uptake and translocation into plant biomass. Lower concentrations of EDTA-extractable Zn in the GWC treatments and a related decrease in plant uptake and BCs could be understood as an increase of Zn complexation onto stable organics and a reduction of Zn available to plant exudates, particularly in the case of GWC amended tailings (Liang *et al.* 2014; Forján *et al.* 2018). Alternatively, the increase in translocation of Zn in the CM amended tailings could indicate a greater propensity of Zn to be mobilised, possibly by the formation of Zn-DOC complexes or by altering the speciation of Zn in the tailing and promoting greater mobilisation (Arnesen and Singh 1998; Clemente *et al.* 2007; Nikoli and Matsi 2011; Mollon *et al.* 2016). It is clear however that despite the elevated pH and high buffering capacity of the tailings a substantial concentration of Zn can be absorbed by plant roots, most likely due to the production of acidifying and chelating reagents into the rhizosphere and the subsequent liberation of Zn from the solid phase.

The plant uptake and translocation of Zn has been discussed earlier in this chapter (*section 7.4.6.3*), and remains relevant to the site at Le Bleymard. Specifically, the elevated concentrations of Zn in plant tissues are probably detrimental to plant functioning, likely producing biotic and oxidative stress. Overall, Zn toxicity to plant development may be significant in the Le Bleymard

tailings due to the exceptionally high total presence of Zn, of which 72% is held in easily dissolved carbonate structures.

7.5.7.5 Implication for Revegetation and Conclusions

The tailings at Le Bleymard present a challenge in terms of revegetation due to the highly calcareous characteristics of the tailings and the largely pH-controlled mobilisation of Cd, Pb and Zn. The calcareous nature of the tailings and alkaline conditions produced unwanted effects such as leaching of NO_3^- and the rapid mineralisation of NH_4^+ (Chapter 6) leading to an overall deficit of N in the soil. Furthermore, the tailing substrate appears to restrict the solubility of plant macro and micro-nutrients, probably via adsorption due to the elevated pH and co-precipitation with Ca and Mg. These factors are also confounded by the dense single grained nature of the tailings, limited weathering processes and conceivable stress from high concentrations of Ca and Mg to vegetation.

An initial solution to this issue would be to attempt to acidify the tailing material, possibly by the incorporation of organic amendments such as pine mulch or bark (Maggard *et al.* 2012; Yan *et al.* 2018), that could aid in the reduction of the tailing pH, reduce adsorption of cations to surfaces and limit the precipitation and leaching effects of P and N species (Cross and Lambers 2017). Another example would be to increase the application rate of fresh CM to 30-40 % w/w, approximately four times as much as used in the current investigation. This approach would potentially also require a degree of irrigation for two primary functions; firstly, to ensure that adequate water is supplied to vegetation, and secondly, to increase the process of hydraulic weathering which should aid in promoting rooting depth and fragmentation of the otherwise compacted structure (Li *et al.* 2015). Such management approaches move away from the idealised *laissez-faire* concepts of ecologically driven remedial systems, and in this case would require a significant amount of organic material to achieve the desired outcome. Fortunately, CM would be in plentiful supply due to the well-established dairy industry in the region. Based on limited long-term experiments in the transformation of skeletal soils and tailings, it could be assumed that a project following these parameters may take around 20 years to establish (Vidal-

Beaudet *et al.* 2012; Santini and Fey 2018; Yuan *et al.* 2018), nevertheless, the benefits of such a scheme have been demonstrated and would likely be successful in the Bleymard tailings in the long term.

However, this study has shown that there is a significant release of Cd, Pb and Zn with decreasing pH (Appendix 1) from the Le Bleymard tailing material, suggesting that improved tailing conditions in terms of nutrient availability would likely be concurrent with a substantial mobilisation of PTE. Indeed, the elevated concentrations of PTE in plant tissues could be a direct result of increased *A.capillaris* root exudates such as organic acids and other chelators in an attempt to enhance nutrient acquisition, with the deleterious consequence of concurrently mobilising PTE (Treeby *et al.* 1989; Mench and Martin 1991; Römheld and Awad 2000; Dakora and Phillips 2002). This would almost certainly negatively impact plant development by increasing PTE burdens beyond the current conditions at a pH of ~7.5, of which uptake of Pb, Cd and Zn into plant biomass is already significant. Furthermore, such an approach would likely promote increased mobility and leaching of PTEs from the tailings into neighbouring environments, and critically, present a significant risk to the adjacent waterbody (Ruisseau de Malavieille) and the downstream environment. This risk is again confounded by the findings of Chapter 4, where the greatest elevations of PTE were generally predicted in the lowest topographical regions of the mine, and directly bordering the waterbody. If a form of organic remediation was to occur as described in the previous paragraph, a barrier system using low-permeable materials such as bentonite or polypropylene geotextiles (Mulligan *et al.* 2001b; Lassabatere *et al.* 2004) would be essential in the remedial approach to prevent groundwater movement of possibly mobilised and chemically active PTE. Furthermore, it would be uncertain if the tolerance of *A.capillaris* to PTE burdens would be exceeded under these conditions, causing vegetative establishment to fail.

It is clear that more research is required into the ecological remediation of PTE-enriched, highly calcareous tailings using a suitable remediation framework. Key barriers to this issue are evident in the lack of suitable sequential extraction

methods in the characterisation of PTE chemical shifts, which can aid in understanding the suitability of organic amendments and plant uptake processes during remedial activities. Furthermore, the identification or production of organic materials that can provide the necessary properties to resolve the contrasting issues of nutrient immobilisation and PTE mobility are also of importance.

7.7 Comparisons of Tyndrum and Le Bleymard Sites

Comparisons between Tyndrum and Le Bleymard untreated and GWC-amended tailing findings are discussed in this section. Cattle manure and SCG are not included in this analysis as they are not directly comparable and were used as site-specific treatments.

7.7.1 Bioaccumulation Coefficients and Translocation Factors

Bio-accumulation coefficients and TFs for *A.capillaris* growing in the un-amended and GWC plots in both sites are presented in Figure 7-21. Bioaccumulation of Cd was significantly higher in untreated Le Bleymard vegetation in comparison to untreated Tyndrum vegetation (Figure 7-21a), with the opposite finding for Pb and Zn. Translocation factors for untreated vegetation were not significantly different between sites for each element (Figure 7-21c).

Agrostis capillaris growing in GWC amended tailings was not found to have significantly different Cd BC between sites, however bio-accumulation for Pb and Zn was significantly higher in the Tyndrum tailings (Figure 7-21b). Similarly to vegetation in the untreated control, no significant differences were observed in the TF for GWC amended tailings across both sites for Cd, Pb or Zn (Figure 7-21d).

Overall, higher BCs were generally observed in vegetation growing at the Tyndrum tailings across both treatments, with the exception of Cd.

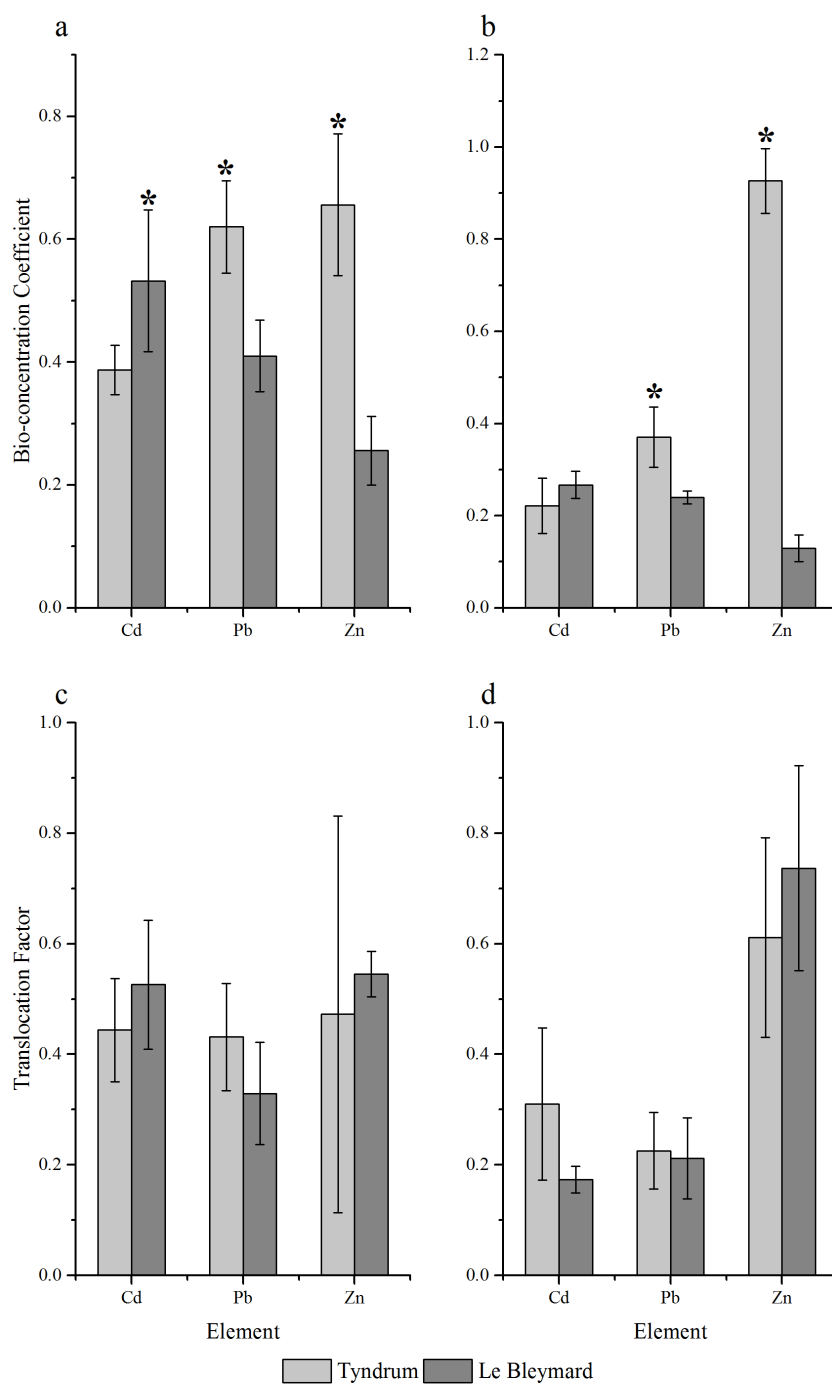


Figure 7-21. Bioaccumulation coefficients for untreated (a) and GWC (b) amended tailings at each site, and translocation factors for untreated tailings (c) and GWC amended tailings (d) at each site. Significant differences between sites for each element are denoted by * $\alpha = 0.05$.

7.7.2 Vegetation biomass and percentage cover

The root and shoot biomasses of vegetation growing in untreated tailings were not significantly different between Tyndrum and Le Bleymard (Figure 7-22), however GWC significantly increased the shoot biomass of *A. capillaris* in Le Bleymard in comparison to Tyndrum. Overall, GWC significantly increased root and shoot biomass at both sites in comparison to vegetation growing on untreated tailings. Vegetation cover however was not significantly different between either treatment or site (Figure 7-23).

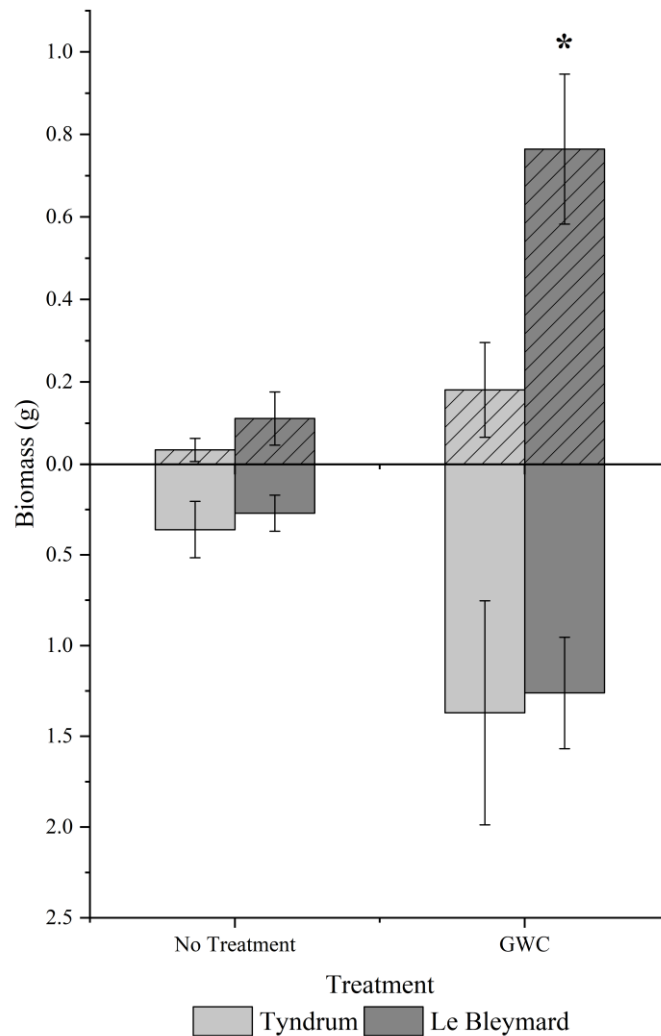


Figure 7-22. Root and shoot biomass of untreated and GWC treated tailings in the Tyndrum and Le Bleymard sites. Significant differences between sites are denoted by * $\alpha = 0.05$.

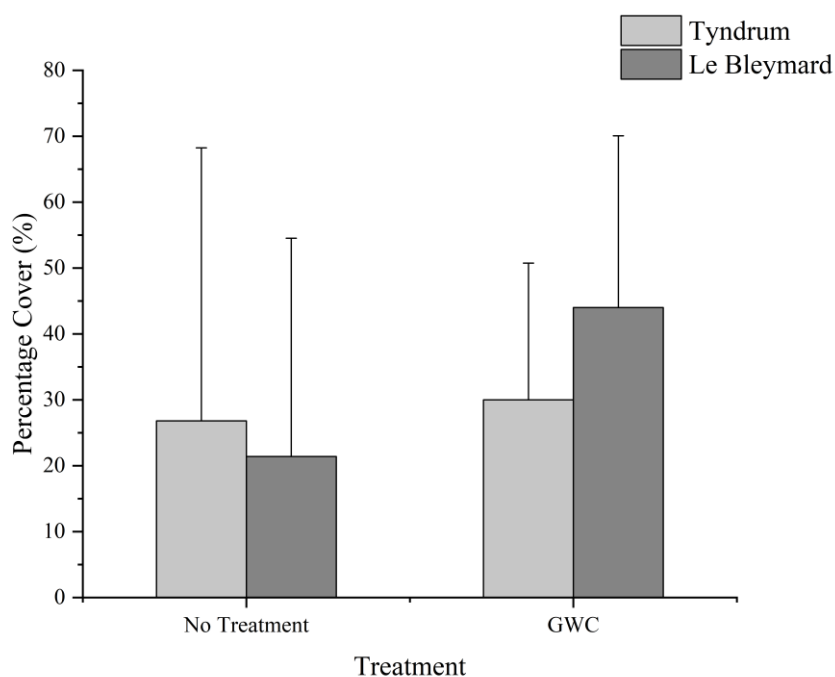


Figure 7-23. Percentage cover of vegetation between Tyndrum and Le Bleymard in untreated and GWC amended tailings.

7.7.3 Tailings Properties

Two-way ANOVA comparing chemical characteristics in untreated and GWC amended tailings across Le Bleymard and Tyndrum demonstrated that, in general, the differences between sites was a greater controlling factor than the incorporation of GWC into the tailings ($p = <0.05$) (Table 7-8). The exception to this finding was the water extractable P and CWEC/HWEC concentrations, where GWC addition to Tyndrum tailings increased concentrations of these properties as compared to the equivalent treatments at Le Bleymard ($p = <0.05$). It was found that Le Bleymard contained significantly higher concentrations of EDTA extractable Cd and Zn in comparison to Tyndrum, where in contrast, EDTA extractable Pb and CaCl_2 extractable Cd, Pb and Zn were significantly higher in the Tyndrum tailings. The tailings at Le Bleymard contained significantly higher concentrations of TC ($p = <0.05$), and a higher baseline pH in comparison to Tyndrum. Concentrations of TN and mineral N were highly variable across sites and treatments, with no significant differences between either.

Table 7-8. Comparison of chemical parameters between untreated and GWC amended tailings at Tyndrum and Le Bleymard. Significant differences between sites after amendment with GWC are highlighted in bold using Two-Way ANOVA ($\alpha = 0.05$).

Parameter	Le Bleymard		Tyndrum	
	No Treatment	GWC	No Treatment	GWC
Total N (%)	0.02 (± 0.01)	0.06 (± 0.03)	0.03 (± 0.03)	0.16 (± 0.08)
NO ₃ ⁻ -N (mg kg ⁻¹)	4.86 (± 3.0)	16.88 (± 17.0)	0.21 (± 0.15)	2.51 (± 3.1)
NH ₄ ⁺ -N (mg kg ⁻¹)	4.4 (± 6.6)	5.53 (± 3.3)	0.84 (± 0.66)	2.17 (± 1.0)
Water soluble P (mg kg ⁻¹)	1.74 (± 0.71)	3.32 (± 1.68)	2.88 (± 2.1)	9.91 (± 3.82)
Total C (%)	8.38 (± 1.7)	10.6 (± 0.88)	0.75 (± 0.67)	3.32 (± 1.6)
CWEC (mg kg ⁻¹)	102 (± 24.1)	102 (± 13.1)	71.9 (± 8.6)	203 (± 53)
HWEC (mg kg ⁻¹)	145 (± 32.9)	367 (± 178)	218 (± 25.9)	823 (± 105)
pH (range)	7.3 – 8.06	7.2 – 8.21	5.75 – 8.00	5.41 – 7.66
CaCl ₂ - Cd (mg kg ⁻¹)	0.01 (± 0.01)	0.04 (± 0.03)	3.8 (± 4.5)	2.4 (± 1.9)
CaCl ₂ - Pb (mg kg ⁻¹)	1.82 (± 0.32)	1.35 (± 0.25)	528 (± 502)	318 (± 289)
CaCl ₂ - Zn (mg kg ⁻¹)	1.16 (± 0.12)	6.87 (± 7.66)	287 (± 217)	321 (± 174)
EDTA - Cd (mg kg ⁻¹)	71.7 (± 10.3)	57.8 (± 14.9)	8.65 (± 7.66)	6.94 (± 5.56)
EDTA - Pb (mg kg ⁻¹)	1,818 (± 981)	934 (± 238)	17,900 ($\pm 11,200$)	18,700 ($\pm 21,300$)
EDTA - Zn (mg kg ⁻¹)	8,052 (± 942)	5,728 (± 697)	2,050 ($\pm 2,600$)	1,950 ($\pm 2,400$)

7.7.4 Discussion

There are obvious contrasts between the field trials undertaken in Tyndrum and in Le Bleymard, with differences in the underlying geological characteristics and environmental conditions arguably having a greater impact on measured characteristic than the addition of GWC. For example, the majority of measured tailing characteristics differ significantly in terms of site location rather than treatment, demonstrating that the addition of GWC had a limited overall impact on tailing properties. This was clear when considering the concentrations of CWEC, TN and water-soluble P, which were significantly lower in the Bleymard tailings amended with GWC as compared with the paired treatment at Tyndrum, despite similar baseline values in the untreated control. The mechanisms behind the lower concentrations have already been discussed, but this comparison further demonstrates the influence of underlying tailing properties on the effectiveness of remediation. In this instance at Le Bleymard, the likely immobilisation of labile C and P through association with Ca^{2+} and Mg^{2+} cations and a high background pH contributing to N leaching.

However, GWC does appear to have some generic capability to mitigate PTE uptake and translocation from contrasting mine tailings. In both sites, GWC reduced the relative bio-accumulation of Cd and Pb, indicating that the incorporation of GWC is useful in suppressing uptake of xenobiotic elements. Contrastingly, Tyndrum Zn bio-accumulation was not decreased in comparison to Le Bleymard. This may have been due to the lower relative concentrations of Zn in the Tyndrum tailings and the tolerance of *A. capillaris* to elevated Zn. Indeed, studies have demonstrated that Zn tolerance in this species can reach over 5,000 mg kg⁻¹ (Houben and Sonnet 2015; Kicińska and Gruszecka-Kosowska 2016), and it is possible that the significantly higher concentrations of total Zn in the Le Bleymard tailings reflect the lower relative bio-accumulation in *A. capillaris*, although this is difficult to determine given the heterogeneity of uptake across treatments and sites. Translocation factors were reduced in both sites for Pb and in Le Bleymard for Cd, indicating that GWC can aid in the reduction of PTE translocation from roots to shoots, possibly

aiding in the reduction of PTE movement in ecological chains (Gramss and Voigt 2014; Aslam *et al.* 2015). Considering *A.capillaris* in particular, the findings in this chapter highlight the inaccuracies of the phytoremedial properties found in Chapter 5, where significantly lower BC and TF factors were assumed. However, this chapter provides excellent evidence of PTE tolerance in this species across diverse environmental conditions, indicating the usefulness of *A.capillaris* as a phytoremediation tool. Contrastingly, *F.ovina* was not found to germinate successfully across the experimental plots at either site, providing evidence that this species is unsuitable for possible future remedial efforts at Tyndrum and Le Bleymard, and perhaps, in mine tailing environments in general. From both chapters it is clear that greenhouse experiments, whilst useful in some respects, are not capable of fully estimating plant responses under field conditions (Vangronsveld *et al.* 2009; Beans 2017).

Concomitantly, GWC amendment promoted vegetative development across both sites in terms of increased plant biomass, with a significant impact on the shoot development of *A.capillaris* in the Le Bleymard tailings. Conversely, no significant difference in surface cover was observed between untreated and GWC amended tailings in either site, suggesting that although the incorporation of GWC may increase aerial biomass it may not aid in the production of a substantial vegetative cap to alleviate surface erosion. It is difficult to determine the controlling factors behind this, and to elucidate if this observation is chiefly a factor of the tailing properties or GWC itself. It could be suggested that the loss of N via leaching or immobilisation processes diminishes a consistent concentration of plant nutrients in the amended tailings, generating an erratic source of viable organic material that can support plants.

It could be concluded that GWC which meet the approximate parameters in Table 6-1 can be used as a useful and widely applicable material to promote vegetative growth and reduce PTE burdens to vegetation, a finding that is not necessarily new due to its prolific use in the remediation of contaminated landscapes (Sheoran *et al.* 2010; Park *et al.* 2011; Ali *et al.* 2013). However, a substantial contribution of the variance in the amended tailings are due to the

underlying site properties, which limits the ability to generalise treatments across disparate mine tailings. Furthermore, the findings in section 7.4.6 provide evidence that more effective organic materials can be used (such as SCG at Tyndrum), indicating that remedial approaches and organic amendments should be based on a case-by case basis.

The calcareous, arid nature of the Bleymard tailings present a challenge in terms of revegetation in comparison the mildly acidic conditions found in Tyndrum. For example, the establishment of suitable root zones are known to take much longer to develop in arid conditions due to the lack of geochemical weathering from rainfall, reducing the potential for non-specialist species to become established (Mendez and Maier 2008). This would be relevant when comparing Tyndrum and Le Bleymard, where changes in physio-chemical characteristics were much more pronounced after a 7-month remediation investigation in the former site. Despite the elevated burdens of PTE, *A.capillaris* demonstrated resilience to these conditions combined with further stressors such as low nutrient content and poor tailing structure. It is difficult to determine if PTE stress or deprivation of nutrients, and water availability were the overriding factor in the poor development in the Bleymard tailings, as the significantly higher concentrations of plant Cd in the Bleymard site in comparison to the Tyndrum tailings may have created substantial physiological stress. This may be particularly pronounced as Cd is a chemical analogue to Zn and therefore is taken up preferentially in comparison to other elements such as Pb (Kaur and Garg 2017; Edelstein and Ben-Hur 2018).

In contrast to the moderate success of *A.capillaris*, the absence of *F.ovina* establishment on both Tyndrum and Le Bleymard provides strong evidence that this species is not suitable for phytostabilisation projects on nutrient poor and PTE enriched mine tailings. It is likely that the PTE concentrations at both sites was the limiting factor for *F.ovina* and were probably linked to a predisposition of greater Pb translocation and possibly oxidative stress to cellular processes in the species. These findings add to the conflicting literature surrounding the species as a phytoremediation candidate (Shimwell and Laurie

1972; Garland and Wilkins 1981; Brown 1995; Yang *et al.* 2005c; Stefanowicz *et al.* 2010b; Bini *et al.* 2017; Lago-Vila *et al.* 2017b) and strengthen the position that *F.ovina* is not suitable for soils or mine tailings with Pb concentrations greater than 10,000 mg kg⁻¹. It is also clear that the use of spiking studies and laboratory trials on expected 'bioavailable' PTE concentrations have limited use in the prediction of phytostabilisation performance in field settings, as seen in the lack of transferability of *F.ovina* from pot to field trials.

Attempts at re-vegetating mine soils have often been reported to fail within 2-3 years, mainly due to the inability of remedial approaches to simulate or reconstruct ecological systems that can fully replicate a normal soil environment (Li *et al.* 2015; Cross and Lambers 2017). This has partly been attributed to a lack of funding for long-term trials and so remedial systems cannot be fully appraised for their suitability over long periods of time (> 10 years). Considering Le Bleymard in particular, the longevity of re-vegetative activities would be critical due to the need for balancing the promotion of a functioning rhizosphere and enhancing nutrient mobility, while co-currently managing the site for exacerbated PTE leaching as a result of acidification. The tailings at Tyndrum would likewise require monitoring, but tailings and environmental conditions at Tyndrum are less challenging than Le Bleymard.

7.8 Chapter Conclusions

A graphical summary of the findings in this chapter are detailed in Figure 7-24.

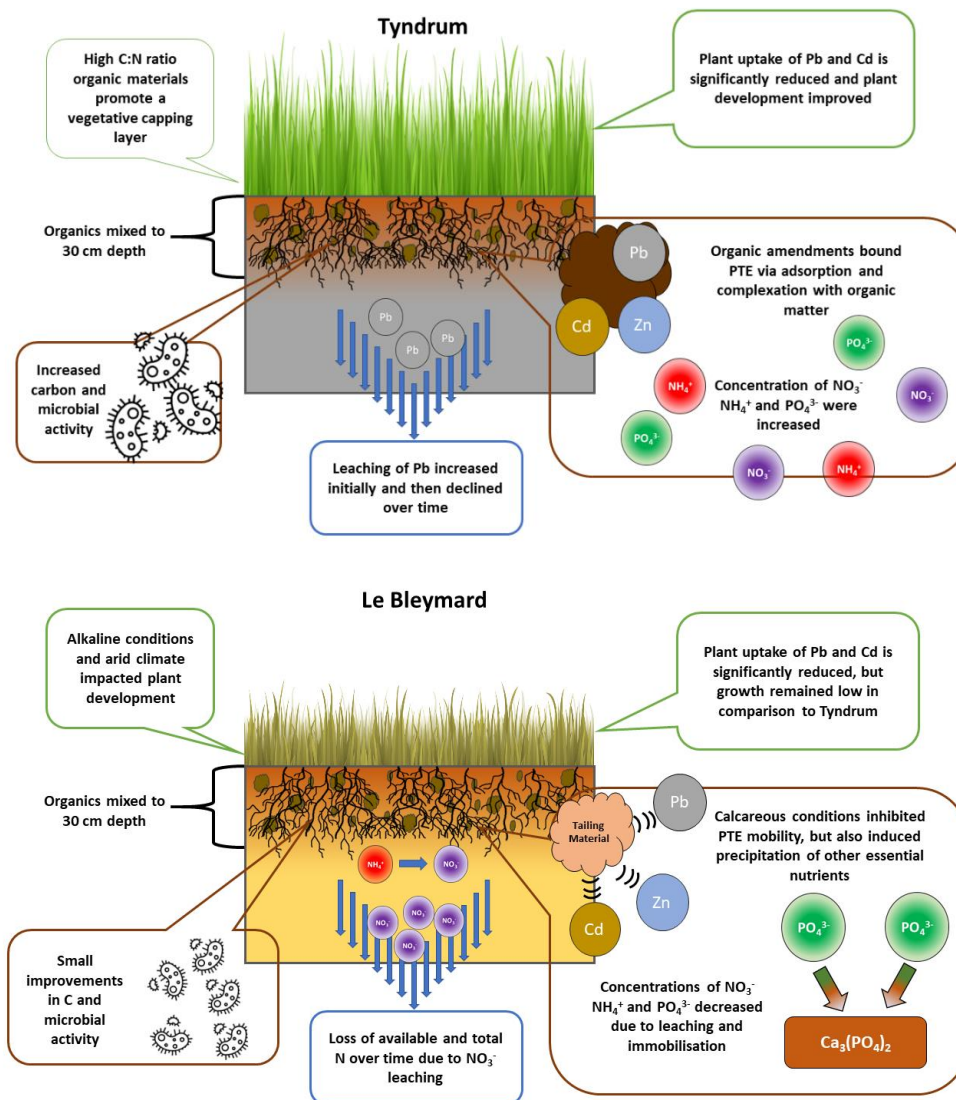


Figure 7-24. Graphical summary of the findings of Chapter 7. Vegetation growth in Tyndrum was highest under spent coffee ground treatments, increasing vegetative establishment, microbial and carbon sources and limiting plant uptake of Cd and Pb, alongside concentrations of weakly associated Pb. The amended Le Bleymard tailings exhibited reduced vegetative cover, loss/immobilisation of plant nutrients and a reduction in the uptake of Cd and Pb by plants

The findings of this chapter have highlighted several important issues considering the ecological remediation of abandoned Pb/Zn mines. Over a 7-month period, the incorporation of organic amendments and the sowing of *A. capillaris* were generally effective in promoting vegetative establishment at both sites to some extent, but was dependent on the amendment in question and

the underlying tailing and environmental characteristics. In general, the incorporation of GWC enhanced the growth and reduced the uptake and transference of PTE to aerial tissues in *A.capillaris* and promoted increases in some tailing parameters, although the magnitude of this effect varied significantly between sites. Significant differences were observed between organic amendments within sites, highlighting the need to carefully select organic materials to maximise the impact of phytostabilisation projects and improve the physio-chemical environment of the tailings.

For Tyndrum it was found that applications of SCG was the most effective approach across several metrics, as this material enhanced vegetative biomass, and immobilised Pb via stable organic complexation. Furthermore, the slow release of N into the tailing system appeared to support vegetative growth and enhanced concentrations of available N into the tailings, suggesting a more sustainable source of plant nutrients than GWC, which showed signs of N loss or unsustainable uptake by vegetation and arguably a less stable immobilisation of Pb.

In consideration of Le Bleymard, the success of vegetative establishment was less pronounced, with limited development of *A.capillaris* across both organic treatments and a higher uncertainty into the immobilisation mechanisms that may have been produced by the incorporation of OM. It was apparent that the environmental conditions at Le Bleymard restricted the potential positive effects provided by the addition of GWC and CM by promoting the loss or immobilisation of plant nutrients due to elevated pH conditions and potential precipitation by Ca, Mg and environmental conditions encountered. Furthermore, despite the widely described sorption of PTE in alkaline conditions, uptake of Cd, Pb and Zn into plant tissues was substantial, particularly when considering Cd and Zn. These effects together likely confounded vegetative development on the tailings and provided difficult conditions for *A.capillaris* to become established. It was found however that GWC in general had a more positive effect on the plant growth at the Bleymard site in comparison to CM and so should be considered for this site.

Finally, it is clear that drawing generalisations when considering remediation are not appropriate when comparing two dissimilar Pb/Zn mines tailings. The underlying geology and local environmental conditions have significant effects on the success of remediation and restrict the ability for a universal ecologically mediated approach to be proposed. That said, the combination of GWC amendment with revegetation using *A.capillaris* does appear to have some generic characteristics that could allow this to be utilised on a range of mine tailings, although targeted amendments and plant species for each case study may be more effective. Future studies involving larger field assessments are essential.

7.8.1 Key findings of the Chapter

The key findings of the chapter are as follows:

1. *Agrostis capillaris* is a highly tolerant species that can withstand elevated concentrations of PTE from a multi-contaminated system, and should be considered when approaching the remediation of abandoned Pb/Zn mines.
2. There is little evidence for the use of *Festuca* as a suitable species for the phytoremediation of abandoned Pb/Zn mines.
3. Environmental and geological conditions are important controlling factors when considering the remediation of abandoned Pb/Zn mines.
4. Calcareous substrates enriched with PTE provide challenging matrices to work with, due to the conflicting issues associated with inducing nutrient mobilisation whilst restricting PTE bioavailability.
5. Organic amendments that contain high C:N ratios may be the most effective in providing suitable immobilisation properties for PTE and the limiting the release of plant nutrients.
6. Laboratory experiments are only partially useful in screening processes and should only be used to inform field trials and explore mechanistic processes, rather than provide prescriptive solutions.

Chapter 8. Conclusions

8.1 Project Conclusions

To address the original objectives in Chapter 1, (*section 1.2*) the following sections aim to summarise the findings of this doctoral thesis and to highlight the contributions made to the wider scientific community on the subject of ecologically mediated metalliferous mine remediation.

8.1.1 The Effect of Tailing Properties on Environmental Risk and Remedial Efforts.

The mine tailings at both Tyndrum and Le Bleymard were found to differ significantly in terms of chemical and physical properties. Concentrations of Pb at Tyndrum were generally dominated in carbonate-held phases in the form of cerussite, ranging from 545 – 40,000 mg kg⁻¹, whereas in Le Bleymard Pb concentrations varied between 1,200 – 26,000 mg kg⁻¹ and were identified to be held predominantly as galena. Furthermore, significantly elevated concentrations of Cd and Zn were observed in Le Bleymard (59 – 427 and 19,000 – 69,000 mg kg⁻¹ respectively) in comparison to Tyndrum, (1.30 – 70 and 130 – 17,000 mg kg⁻¹ respectively), attributed to the extensive presence of smithsonite and minrecordite in the Le Bleymard tailing heaps. The distributions of total PTE varied between sites, with clear observations of Pb, Zn and Cd accumulation in the lower western area of the Bleymard tailings and the highly heterogeneous distribution of these elements at Tyndrum. It was found that the distribution of Pb was intrinsically linked to particle size at Le Bleymard, with an observed migration of small, Pb enriched particulates from the upper area of the mine to the lower sections and deposition into a nearby watercourse. In contrast, no observable environmental gradient at Tyndrum was observed for PTE transportation. Other key differences were observed in terms of tailing pH (~5.5 at Tyndrum and ~7.5 at Le Bleymard), K_d values, and environmental conditions.

Despite the higher concentrations of Cd and Zn, and the elevated concentrations of Pb at Le Bleymard, K_d values were significantly lower for these elements in comparison to Tyndrum ($p = <0.001$), indicating a lower

relative latent risk of bioavailability and mobility of PTE from the mine tailings into soil and water environments. However, due to the relationship of PTE enrichment and particle size at Le Bleymard combined with the arid nature of the tailings, aeolian routes of transportation and subsequent inhalation of PTE enriched particulates was identified as a significant risk at this site. Overall, the concentrations of PTE at the Tyndrum site was considered to be more immediately available and significantly more mobile than that of Le Bleymard, however, the exceptionally elevated concentrations of PTE at Le Bleymard present a serious latent risk to human health if inhalation or desorption processes occurred.

The underlying physio-chemical properties of the tailings had a substantial impact on the effect of organic amendment incorporation and plant growth. In the mildly acidic tailings at Tyndrum the introduction of organic ligands and soluble Fe-compounds enhanced the mobility of Pb in the Tyndrum material, whilst decreasing Cd and Zn mobility as a result of increased pH and potential immobilisation via precipitation reactions. Over a longer period, substantial changes in PTE fractionations were recorded upon the addition of GWC and SCG, where a significant decrease in weakly associated Pb was observed with the latter material. This appears to be mainly induced by the dissolution of weakly held PTE and the subsequent complexation with Fe/Mn oxy-hydroxides and stable organic compounds. Furthermore, significant improvements in microbial respiration, biomass, N and P availability and carbon reserves were generally recorded upon the incorporation of organic amendment to the Tyndrum tailings, again, specifically in reference to SCG. The coupled alterations in PTE fractionation and tailing nutrient content increased vegetation growth of *A.capillaris* and aided in root and surface establishment of field investigations.

In contrast, the highly calcareous nature of the Le Bleymard tailings reduced possible PTE leaching effects from organic amendments, probably due to the increased pH of the tailings and strong adsorption or precipitation effects of potentially liberated PTE. However, the calcareous substrate also elicited the

leaching of NO_3^- , the rapid mineralisation of NH_4^+ and the immobilisation of available P and labile carbon sources. This holistic immobilisation and leaching effect was likely detrimental to the growth of *A.capillaris* by reducing the availability of essential plant nutrients, therefore diminishing the suitability of ecologically enhanced remediation to be attained on this site. Clearly, the underlying geochemical conditions, and importantly pH and buffering capacity have a substantial impact on the success and potential revegetation of abandoned mine tailings.

8.2 Important Properties of Plants and Organic Amendments when Considering Ecologically Driven Remediation.

The screening of potential phytoremediation species is an important part in determining their suitability. It was found that species from the *Poaceae* family were generally better in generating root systems and surface cover than several of the *Brassicales* which were found to generate low rooting biomass and increase of aerial biomass production. When comparing laboratory studies to field investigations it was evident that the translation of findings was not evident, with the previously assumed low bioconcentration co-efficients and translocation factors observed in laboratory settings found to be erroneous under field conditions, particularly in the case of *F.ovina*. Clearly, pot studies do not allow for accurate inferences to be made of plant PTE uptake and should be used with caution if attempting to extrapolate characteristics to real situations. Despite this, *A.capillaris* was demonstrated to be an intrinsically resilient species under challenging environmental and chemical conditions, and so should be considered if phytoremediation of either mine sites was to occur. It can be concluded that prospective plant species should exhibit dense surface cover and root systems, with limited translocation of PTE to the aerial components. Furthermore, species that have been identified as having inherent resilience to challenging conditions would be preferential, as it avoids the expensive requirement to cultivate and propagate specialist ecotypes.

The selection process for identifying organic amendments to incorporate into mine tailings are not as obvious. Humified organic materials such as GWC

appear to provide stable sources of carbon, immediately available nitrate and reduce the risks of enhancing PTE leaching. However, these properties may not be desirable in skeletal tailing systems, where sustainable sources of plant nutrients and the stimulation of microbial systems are required to overcome the nutrient deficient conditions and facilitate ecological recovery. This is specifically important for calcareous mine tailings, where high buffering capacities and elevated pH diminish the potential of cationic mobility, elicit N loss and P precipitation, potentially limiting the usefulness of humified materials such as GWC.

Contrastingly, biologically active organic materials such as SM, CM and SCG have been demonstrated to provide a greater enhancement of microbial respiration and nutrient availability in the short term, and in the case of SCG, over longer periods of time. Furthermore, it appears that incorporating organic amendments containing high proportions of soluble C can aid in the immobilisation of Pb over the short-medium term and should be considered as an approach for binding Pb to stable organic constituents over longer periods of time. However, the presence of soluble organic ligands and Fe compounds demonstrably pose a risk to the mobility of Pb from the Tyndrum tailings, and so these materials should be considered with leaching risks in mind.

Considering the results of each chapter holistically, it would be suggested that the most appropriate organic material for the tailing site at Tyndrum should exhibit characteristics similar to SCG; a high C:N ratio, a range of carbon compounds as microbial energy sources and PTE binding, and a low concentration of soluble Fe. It is suggested that an application of $\sim 200 \text{ t ha}^{-1}$ of such a material should aid in the establishment of vegetation and reduce PTE availability. Considering Le Bleymard, the identification of a suitable material is less clear, with conflicting requirements to reduce the pH of the tailing environment to enhance N retention and plant acquisition of essential cations, with the concurrent risk of inducing significant PTE mobility as a result of an increase in acidic conditions. It is possible that higher applications of organic amendments than used in this thesis could facilitate tailing improvement for

vegetation, however this would require investigation in both laboratory and carefully monitored field settings.

8.3 Summary Conclusions

This thesis has attempted to expand our understanding relating to ecologically driven remediation approaches in abandoned Pb/Zn mines. Overall the investigations included in the work explored the geochemical characteristics of two contrasting mining sites and the effectiveness of a range of organic materials and plant species in the promotion of microbial regeneration, changes in PTE behaviour and establishment of vegetation. Diverse observations in terms of geochemical properties and related risks were identified for both sites, with the Tyndrum tailings identified as presenting an immediate risk to the surrounding environment due to higher PTE mobility, and the Le Bleymard site establishing a latent issue resulting from the high sorption of PTE to tailing material.

Considering the findings holistically, ecologically mediated remediation using organic amendments and *A.capillaris* as a vegetative cap could be achieved on the Tyndrum mine using SCG or an organic material with similar chemical properties. In contrast, this approach of restoration was identified to be challenging in the calcareous tailings found at Le Bleymard, where the elevated pH and high buffering capacity of the tailing material limited the supporting effect of organic amendments in the establishment of vegetation. Overall, it is clear that these approaches can, and should be brought forward into larger, long term field trials or commercial applications for sites similar to the Tyndrum tailings. However, more research is required regarding calcareous mine tailings.

8.3.1 Key Findings

Key findings and contributions to the wider literature are:

- The applicability of geo-statistical interpolation in the characterisation of abandoned Pb/Zn mines and the usefulness in identifying transport mechanisms of PTE to the wider environment.

- The identification of *A.capillaris* as an excellent candidate for the revegetation of abandoned Pb/Zn mines.
- The conclusion that *F.ovina* is not a suitable candidate for the for the revegetation of abandoned Pb/Zn mines.
- The advancement of knowledge regarding PTE mobilisation, transformation and fate in mine tailings amended with a range of organic materials, with specific reference to the mobilisation vectors of soluble carbon and Fe compounds.
- The demonstration of organic amendments in the sequestration and alteration of PTE fractionation in field conditions in acidic to neutral mine tailings.
- The use of the BCR-1 and EDTA extractions as useful indicator of plant uptake of PTE in acidic/neutral and calcareous mine tailings respectively.
- The identification of key factors when considering the remediation of abandoned mine tailings, with specific reference to managing the availability of plant nutrients and carbon whilst considering risks from enhanced PTE mobilisation.

8.4 Recommendations for Future Work

Future investigations following from this thesis should ideally focus on the expansion of field trials at both mining sites, in terms of the length of time and in the size of application. Furthermore, a more sophisticated plot design would aid in the facilitation of long-term monitoring and should include an effective system of leachate and runoff collection, gas sampling and vegetation monitoring. Moreover, as this thesis has focused mainly on geochemical

changes, investigations centred around impacts to microbial communities and biological parameters would support the concept of ecologically driven remedial approaches and should be considered. Considering Le Bleymard specifically, there is a lack of information relating to the current human and environmental impacts from tailing material transported from the site, providing scope for several studies under this remit. Furthermore, the development of analytical procedures relating to calcareous tailings should be considered due to the difficulties encountered in assessing several tailing parameters throughout this thesis.

Specifically, the following recommendations for future projects are described:

- The development of a sequential extraction that can be used in alkaline or calcareous soil systems to inform investigators of PTE fractionation and PTE risk.
- Quantifying the bio-accessibility and inhalation risk of PTE enriched tailing material from the Le Bleymard tailings to human health.
- Identifying the extent of PTE transportation from Le Bleymard to the wider environment via water courses, utilising ICP-MS and isotope discrimination to trace material from the mine to a receptor point.
- The establishment of larger field trials at both sites (3-5 years), with specific focus on the monitoring of soil formation changes, biological parameters and PTE behaviour.
- The identification of a suitable material to overcome the issues relating to nutrient issues at the Le Bleymard site whilst managing the risks of enhancing PTE release.

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Appendix 1. PTE desorption and pH in the Le Bleymard Tailings

To elucidate if changes in pH had an effect on the release of Cd, Pb and Zn from the tailings, a 5 g sample of air dried and ball milled tailings from the homogenised material in Chapter 6 was placed in 20 ml of deionised water in 50 ml centrifuge tubes and buffered between a pH range of 4 – 8 in 0.5 intervals using concentrated nitric acid (68%, Aristar®, VWR). The samples were placed in an end-over-end shaker for 30 minutes at 30 rpm, centrifuged at $8,000 \times g^{-1}$ and the supernatant passed through a $0.45 \mu m$ filter. All analysis was conducted at ambient temperature (16-18°C).

The collected supernatant was analysed for Cd, Pb and Zn via ICP-OES (Chapter 3, section 3.7.17). Collected supernatants were analysed in duplicate with three replicate samples at each pH interval.

It was found that as pH decreased increasing concentrations of Cd, Pb and Zn were released into solution, with a plateau in concentration around pH 6 – 6.5 for all elements (Figure A 1), demonstrating that pH is a significant controlling factor in the release of PTE from the Le Bleymard tailings.

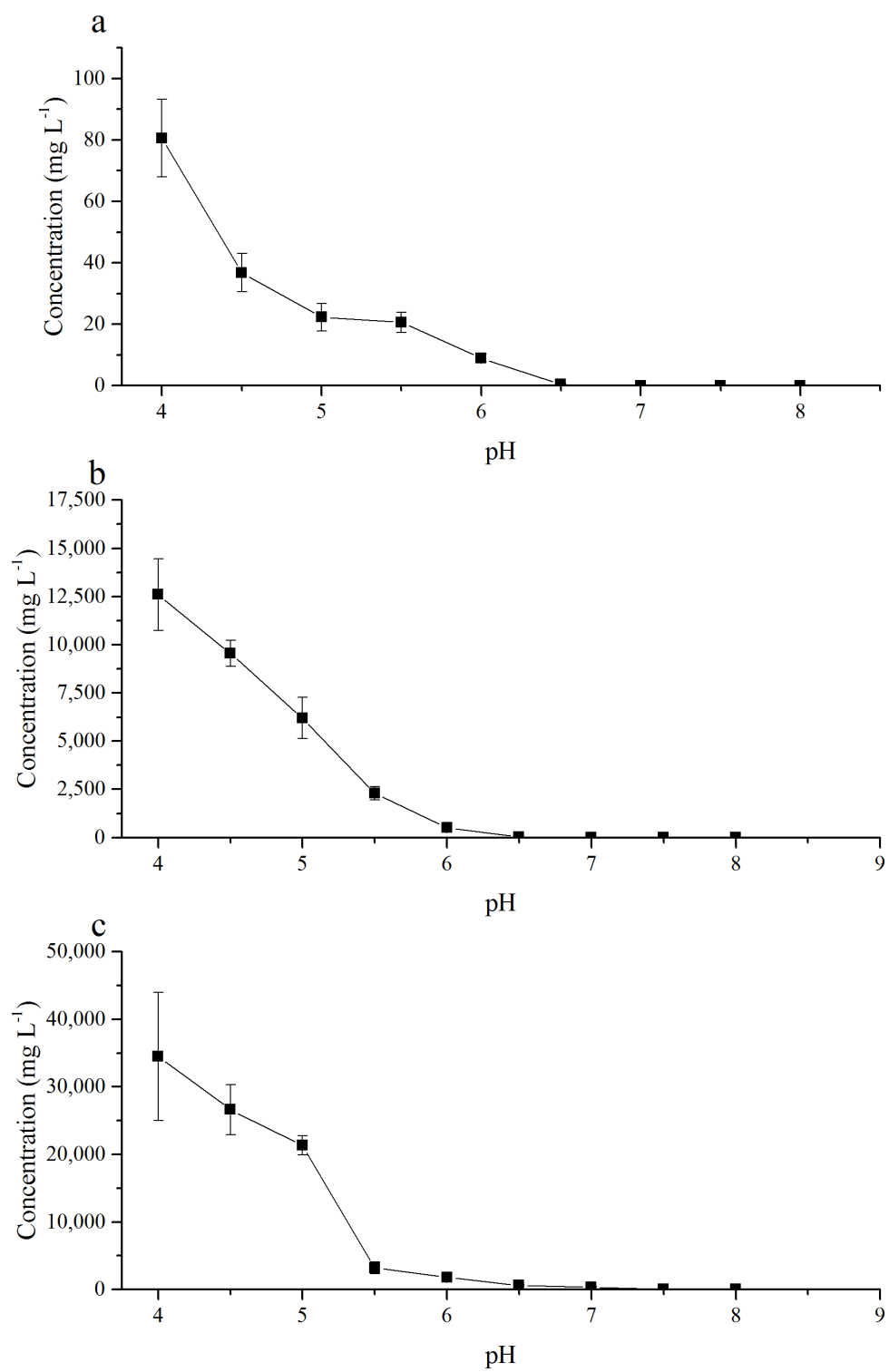


Figure A 1. Concentrations of PTE as a function of pH for Cd (a), Pb (b), and Zn (c) $n = 3$.

Appendix 2. BCR-Protocol and Le Bleymard Tailings

The application of the BCR-protocol was not found to be appropriate to the Le Bleymard tailing material due to the buffering capacity of the tailing material on the analytical solution. The BCR procedure states that the analytical solution should have a pH of < 2 to ensure that target elements remain in solution and prevent re-adsorption onto solid surfaces, therefore reducing the underestimation of PTE in a particular fraction. However, the pH of the first two fractions of the procedure were above pH 4 (Table A 1). Based on the increased absorption of PTE, and in particular Pb, to solid surfaces at pH conditions greater than 4 (Alloway 2012) it would be expected that re-adsorption would be occurring, therefore the reliability of the procedure is compromised.

Table A 1. pH of the analyte solution in the weakly associated and reducible fractions of the Tyndrum and Le Bleymard groups, BCR-701 reference material and analytical blanks. n = 8.

BCR-Fraction	Tyndrum Samples	Le Bleymard Samples	BCR-701 CRM	Analytical Blanks
Weakly associated	2.66 (± 0.16)	5.27 (± 0.3)	2.91 (± 0.04)	2.45 (± 0.06)
Reducible	2.31 (± 0.09)	4.79 (± 0.2)	1.07 (± 0.07)	0.87 (± 0.07)

Appendix 3. Eh values of collected leachate from the Tyndrum and Le Bleymard groups.

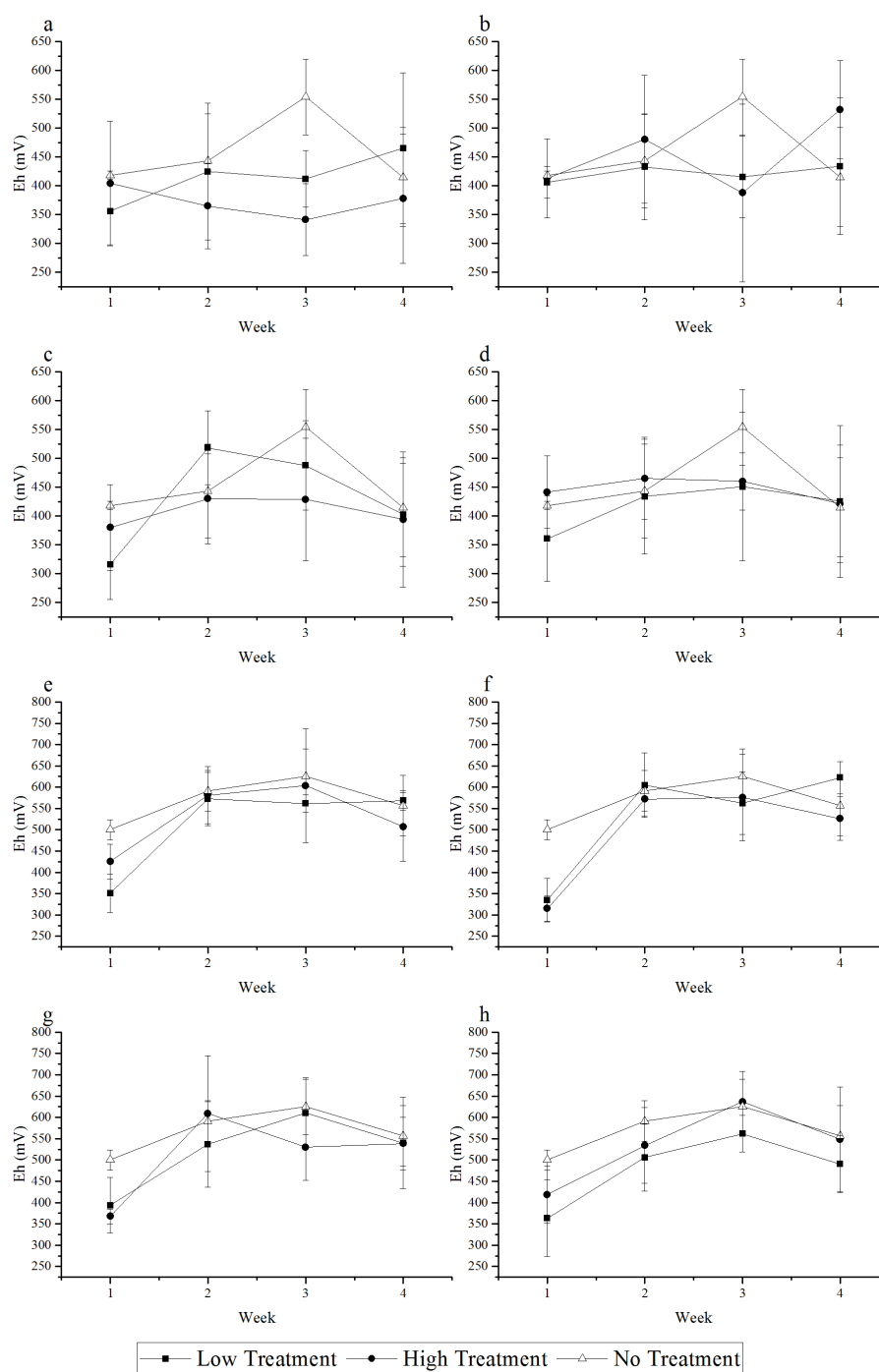


Figure A 2. Eh of collected leachate in CM (a), SM(b), GWC (c), SCG (d) in the Tyndrum Group and in CM (e), SM (f), GWC (g) and SCG (h) from the Le Bleymard column group. n = 4.

Appendix 4. R script for Principal Component Analysis

```
install.packages("devtools")

library("devtools")

install_github("vqv/ggbiplot")

library("ggbiplot") # Installation of ggbiplot packages to visualise data

character_vars <- lapply(data, class) == "character" data[, character_vars] <- lapply(data[,character_vars], as.factor) # Conversion of text data to factor data for grouping in the PCA

str(data) # View the data to ensure factoring has worked

pca<- prcomp(data [2:12],scale.=TRUE) # Application of Principal Component Analysis

g<-ggbiplot(pca, obs.scale=1, var.scale=1, ellipse=TRUE, circle=TRUE, groups= data $Treatment, ellipse.prob = 0.68,) # Production of biplot to visualise data, with grouping confidence intervals of 1 standard deviation (68%)

g<-g+scale_color_discrete(name="")

g+theme(panel.background = element_rect(fill = 'white', colour = 'white')) # applying white background and colour scheme for improved clarity.
```

Appendix 5. Principal Component Analysis for Individual Treatments

Tyndrum Biplots for Leaching Columns

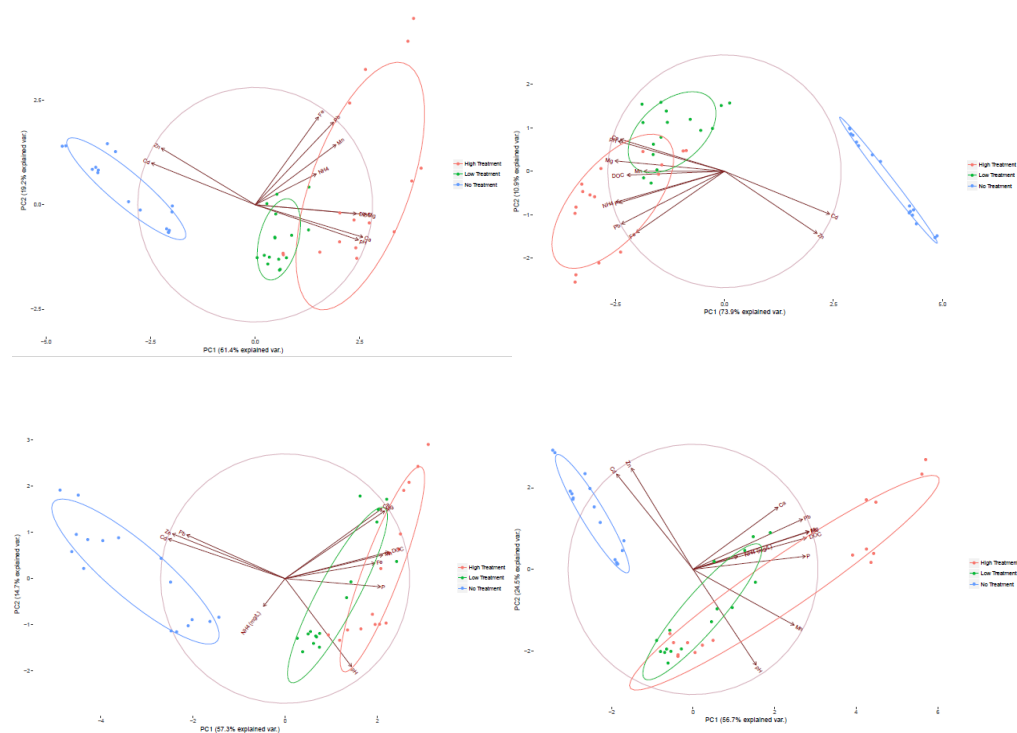


Figure A 3. Biplots of leachate properties in the Tyndrum group. a) cattle manure, b) sheep manure, c) compost and d) spent coffee grounds.

Tyndrum Cattle Manure Principal Component Analysis

Table A 2. Principal components 1-5 for cattle manure treated Tyndrum tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC 5
Eigenvalue	6.9557	1.9871	0.9323	0.6169	0.1961
Proportion	0.632	0.181	0.085	0.056	0.018
Cumulative	0.632	0.813	0.898	0.954	0.972

Table A 3. Eigen vectors for cattle manure treated Tyndrum tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
----------	-----	-----	-----	-----	-----

pH	0.326	0.247	-0.071	-0.065	-0.325
DOC	0.334	0.065	0.236	0.487	0.040
NH₄⁺	0.196	-0.150	-0.797	0.300	-0.381
Ca	0.341	0.228	0.030	0.043	0.334
Cd	-0.325	-0.289	-0.024	0.352	0.227
Fe	0.218	-0.514	0.266	-0.273	-0.301
Mg	0.360	0.084	0.067	0.296	0.274
Mn	0.263	-0.320	-0.399	-0.347	0.629
Pb	0.268	-0.481	0.182	-0.105	-0.118
Zn	-0.289	-0.378	0.025	0.421	0.083
P	0.347	-0.170	0.183	0.270	-0.046

Tyndrum Sheep Manure Principal Component Analysis

Table A 4. Principal components 1-5 for sheep manure treated Tyndrum tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC 5
Eigenvalue	8.1320	1.2022	1.0718	0.3464	0.0820
Proportion	0.739	0.109	0.097	0.031	0.007
Cumulative	0.739	0.849	0.946	0.977	0.985

Table A 5. Eigen vectors for sheep manure treated Tyndrum tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	0.319	-0.235	-0.208	0.176	0.718
DOC	0.292	0.030	-0.462	0.346	-0.480
NH₄⁺	0.326	0.242	-0.144	0.145	0.204
Ca	0.312	-0.251	0.309	0.086	-0.393
Cd	-0.316	0.336	0.064	0.363	-0.030
Fe	0.265	0.482	0.178	-0.549	0.097
Mg	0.328	-0.081	0.216	0.304	0.075

Mn	0.241	0.001	0.662	0.283	0.032
P	0.316	0.248	-0.310	0.065	-0.062
Pb	0.309	0.414	0.053	-0.117	-0.125
Zn	-0.278	0.488	0.086	0.444	0.148

Tyndrum Compost Principal Component Analysis

Table A 6. Principal components 1-5 for compost treated Tyndrum tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC 5
Eigenvalue	6.3006	1.6133	1.0574	0.7572	0.4537
Proportion	0.573	0.147	0.096	0.069	0.041
Cumulative	0.573	0.719	0.816	0.884	0.926

Table A 7. Eigen vectors for compost treated Tyndrum tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	0.213	0.556	0.066	-0.258	-0.398
DOC	0.332	-0.169	-0.027	-0.185	-0.457
NH₄⁺	-0.069	0.175	-0.838	-0.451	0.151
Ca	0.310	-0.442	-0.151	0.065	-0.112
Cd	-0.373	-0.251	0.040	-0.109	-0.054
Fe	0.287	-0.098	0.311	-0.575	-0.062
Mg	0.319	-0.427	-0.157	0.013	-0.100
Mn	0.313	-0.149	-0.248	0.175	0.262
P	0.306	0.051	0.271	-0.275	0.711
Pb	-0.315	-0.277	0.099	-0.447	0.037
Zn	-0.360	-0.283	0.041	-0.206	-0.066

Tyndrum Spent Coffee Grounds Principal Component Analysis

Table A 8. Principal components 1-5 for spent coffee ground treated Tyndrum tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC 5
Eigenvalue	6.2721	2.3704	1.0746	0.6856	0.2897

Proportion	0.570	0.215	0.098	0.062	0.026
Cumulative	0.570	0.786	0.883	0.946	0.972

Table A 9. Eigen vectors for spent coffee ground treated Tyndrum tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	0.192	-0.477	0.185	-0.342	-0.537
DOC	0.364	0.158	-0.216	0.122	0.110
NH₄⁺	0.141	0.051	0.865	-0.083	0.434
Ca	0.271	0.357	-0.174	-0.475	0.287
Cd	-0.285	0.421	0.155	0.197	-0.167
Fe	0.374	0.174	0.068	0.164	-0.195
Mg	0.367	0.211	-0.085	-0.133	-0.061
Mn	0.241	-0.346	-0.009	0.677	0.213
P	0.359	0.055	0.256	0.036	-0.402
Pb	0.354	0.239	-0.065	0.230	-0.097
Zn	-0.263	0.430	0.186	0.200	-0.377

All Tyndrum Treatment Groups Principal Component Analysis

Table A 10. Principal components 1-5 for all treatment groups in the Tyndrum tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC 5
Eigenvalue	5.1457	2.0525	1.5279	1.1026	0.5457
Proportion	0.468	0.187	0.139	0.100	0.050
Cumulative	0.468	0.654	0.793	0.894	0.943

Table A 11. Eigen vectors for all treatment groups in the Tyndrum tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	0.346	-0.102	0.002	0.477	0.136
DOC	0.236	0.024	-0.514	-0.355	0.464
NH₄⁺	0.314	0.341	0.263	0.149	0.350
Ca	0.172	-0.406	0.409	-0.454	-0.075

Cd	-0.341	0.386	0.059	-0.230	0.127
Fe	0.296	0.351	0.117	-0.076	-0.675
Mg	0.348	-0.203	0.174	-0.430	0.144
Mn	0.215	0.010	-0.597	0.013	-0.214
P	0.333	0.338	0.266	0.156	0.240
Pb	0.349	0.307	-0.150	-0.273	-0.177
Zn	-0.304	0.431	0.051	-0.276	0.106

Le Bleymard Biplots for Leaching Columns

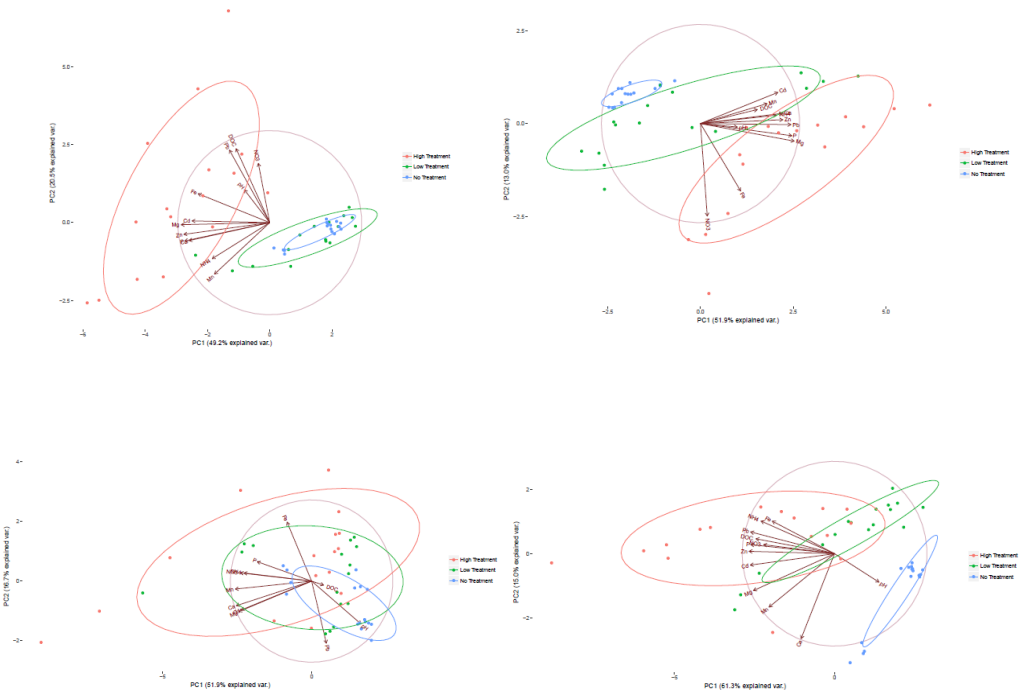


Figure A 4. Biplots of leachate properties in the Le Bleymard group. a) Cattle manure, b) sheep manure, c) compost and d) spent coffee grounds.

Le Bleymard Cattle Manure Principal Component Analysis

Table A 12. Principal components 1-5 for cattle manure treated Le Bleymard tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	5.9008	2.4613	1.3566	1.0047	0.4769
Proportion	0.492	0.205	0.113	0.084	0.040
Cumulative	0.492	0.697	0.810	0.894	0.933

Table A 13. Eigen vectors for cattle manure treated Le Bleymard tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	0.114	-0.227	-0.716	0.117	0.193
DOC	0.150	-0.511	0.055	-0.433	-0.048
NO₃⁻	0.051	-0.409	0.446	0.478	-0.106
NH₄⁺	0.257	0.249	0.404	-0.143	0.510
Ca	0.361	0.124	-0.273	-0.028	-0.290
Cd	0.347	-0.012	-0.009	-0.221	0.387
Fe	0.320	-0.200	0.083	0.340	-0.314
Mg	0.396	0.015	-0.123	0.187	0.048
Mn	0.247	0.356	0.096	-0.359	-0.569
P	0.380	0.127	0.088	0.103	-0.020
Pb	0.180	-0.502	0.090	-0.396	-0.034
Zn	0.384	0.081	-0.008	0.232	0.171

Le Bleymard Sheep Manure Principal Component Analysis

Table A 14. Principal components 1-5 for sheep manure treated Le Bleymard tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	6.2333	1.5574	1.2633	0.9917	0.6407
Proportion	0.519	0.130	0.105	0.083	0.053
Cumulative	0.519	0.649	0.754	0.837	0.891

Table A 15. Eigen vectors for sheep manure treated Le Bleymard tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	0.149	0.028	-0.167	0.882	0.090
DOC	0.229	-0.113	-0.382	0.003	-0.828
NO ₃ ⁻	0.028	0.747	-0.203	-0.062	0.106
NH ₄ ⁺	0.307	-0.068	0.389	0.031	-0.228
Ca	0.332	-0.083	0.053	-0.267	0.210
Cd	0.313	-0.252	-0.148	-0.216	0.095
Fe	0.162	0.545	0.354	-0.095	-0.330
Mg	0.379	0.142	-0.108	-0.042	0.176
Mn	0.269	-0.161	0.560	0.120	0.005
P	0.369	0.098	0.125	0.183	0.114
Pb	0.365	0.009	-0.257	0.040	0.079
Zn	0.333	-0.031	-0.277	-0.199	0.178

Le Bleymard Compost Principal Component Analysis

Table A 16. Principal components 1-5 for compost treated Le Bleymard tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	6.5264	1.9278	1.2584	0.8115	0.5775
Proportion	0.544	0.161	0.105	0.068	0.048
Cumulative	0.544	0.705	0.809	0.877	0.925

Table A 17. Eigen vectors for compost treated Le Bleymard tailings in Chapter 6

Variable	PC1	PC2	PC3	PC4	PC5
pH	-0.230	-0.393	-0.022	0.392	-0.452
DOC	-0.058	-0.029	-0.761	0.410	0.286
NO ₃ ⁻	0.335	0.129	-0.233	-0.197	-0.410

NH₄⁺	0.316	0.127	-0.149	-0.275	-0.486
Ca	0.370	-0.179	0.049	0.177	0.052
Cd	0.350	-0.223	-0.017	-0.191	0.217
Fe	0.099	0.543	-0.381	-0.022	0.044
Mg	0.360	-0.234	0.062	0.172	0.074
Mn	0.370	-0.031	-0.014	0.120	0.105
P	0.252	0.209	0.166	0.622	-0.319
Pb	-0.056	-0.544	-0.403	-0.253	-0.230
Zn	0.360	-0.200	0.053	-0.020	0.293

Le Bleymard Spent Coffee Grounds Principal Component Analysis

Table A 18. Principal components 1-5 for spent coffee ground treated Le Bleymard tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	7.3607	1.7970	1.1014	0.7019	0.3270
Proportion	0.613	0.150	0.092	0.058	0.027
Cumulative	0.613	0.763	0.855	0.913	0.941

Table A 19. Eigen vectors for spent coffee ground treated Le Bleymard tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	-0.177	0.230	0.568	-0.646	-0.025
DOC	0.313	-0.120	0.138	0.068	-0.725
NO₃⁻	0.283	-0.073	-0.379	-0.459	0.395
NH₄⁺	0.293	-0.263	-0.268	-0.329	-0.072
Ca	0.133	0.685	0.052	0.066	-0.012
Cd	0.336	0.092	-0.098	-0.034	-0.234
Fe	0.248	-0.263	0.418	0.425	0.387
Mg	0.323	0.298	0.177	-0.036	0.105
Mn	0.261	0.431	-0.264	0.238	0.085

P	0.334	-0.073	0.285	-0.020	0.289
Pb	0.334	-0.176	0.271	-0.063	-0.073
Zn	0.341	-0.019	-0.026	-0.103	-0.036

All Le Bleymard Treatment Groups Principal Component Analysis

Table A 20. Principal components 1-5 for all treatment groups in the Le Bleymard tailings in Chapter 6.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	4.7615	2.2830	1.5267	1.0200	0.6876
Proportion	0.397	0.190	0.127	0.085	0.057
Cumulative	0.397	0.587	0.714	0.799	0.857

Table A 21. Eigen vectors for all treatment groups in the Le Bleymard tailings in Chapter 6.

Variable	PC1	PC2	PC3	PC4	PC5
pH	0.037	0.192	-0.542	-0.507	0.408
DOC	0.224	-0.442	-0.213	-0.257	-0.205
NO₃⁻	0.093	0.188	0.486	-0.519	-0.517
NH₄⁺	0.319	0.277	-0.196	0.318	-0.333
Ca	0.312	0.250	0.281	-0.300	0.315
Cd	0.346	-0.134	0.342	-0.059	0.307
Fe	0.237	-0.372	0.027	0.140	-0.113
Mg	0.392	0.220	-0.136	-0.086	-0.034
Mn	0.323	0.251	0.106	0.280	0.107
P	0.368	0.237	-0.278	0.143	-0.173
Pb	0.288	-0.404	-0.217	-0.222	-0.194
Zn	0.298	-0.324	0.187	0.193	0.356

Appendix 6. BCR-Procedure Analysis of Means Outputs (ANOM)

Chapter 6 ANOM for Tyndrum tailing Cd, Pb and Zn

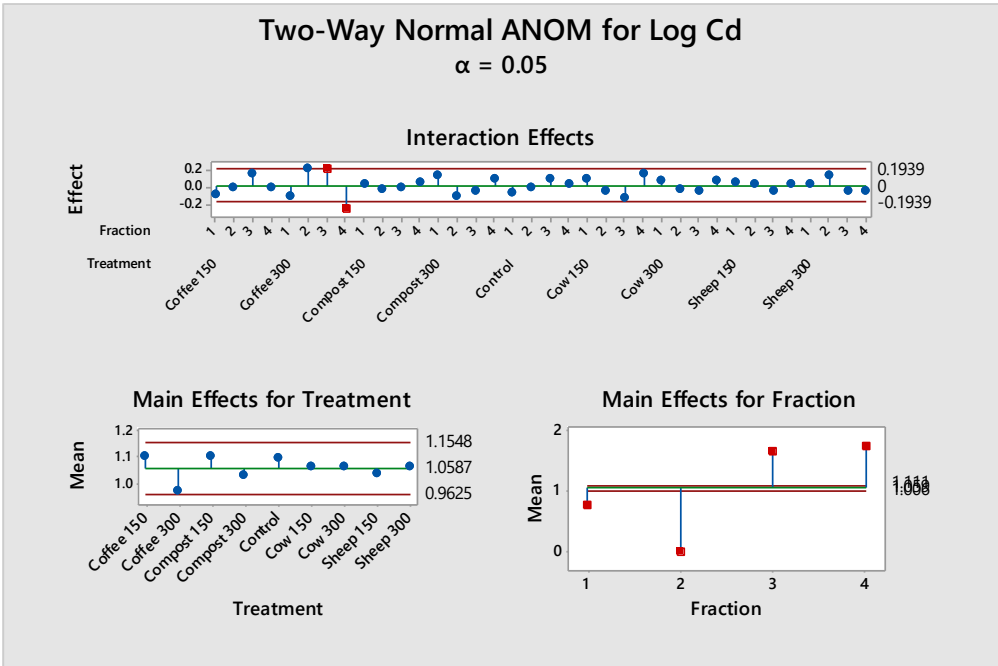


Figure A 5. Two-way normal analysis of means for log transformed Cd BCR fractions applied to the Tyndrum columns in Chapter 6.

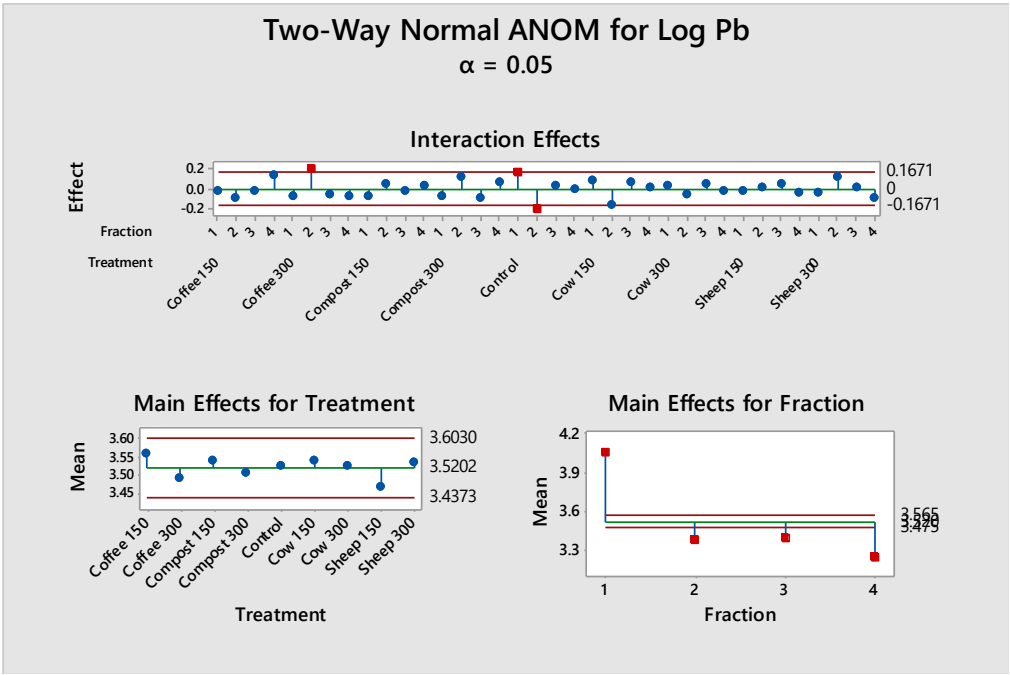


Figure A 6. Two-way normal analysis of means for log transformed Pb BCR fractions applied to the Tyndrum columns in Chapter 6.

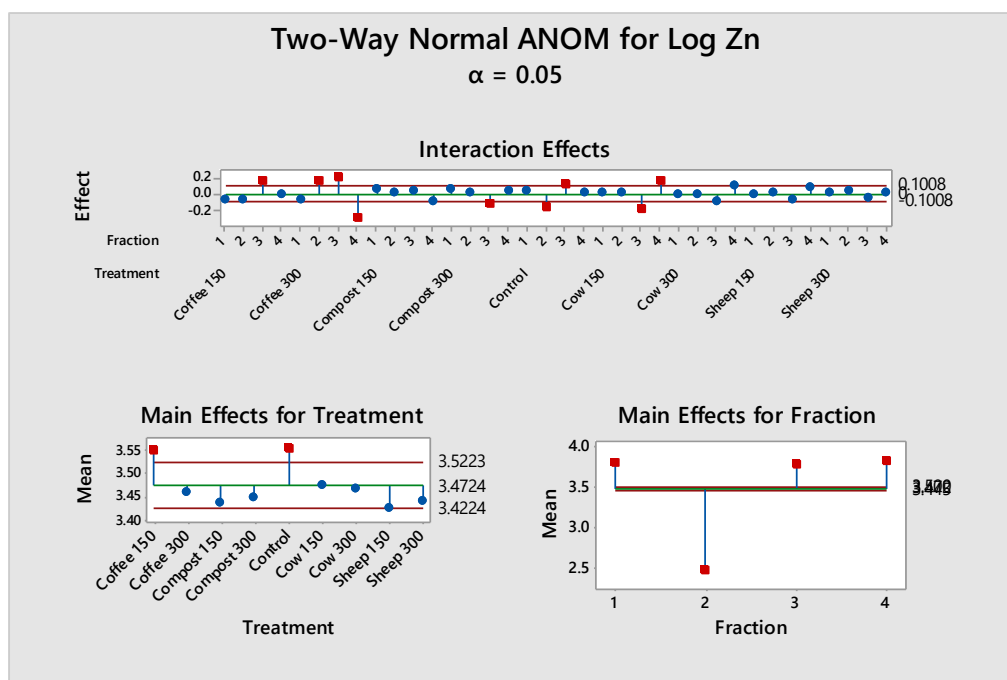


Figure A 7. Two-way normal analysis of means for log transformed Zn BCR fractions applied to the Tyndrum columns in Chapter 6.

Chapter 7 ANOM for Tyndrum tailing Cd, Pb and Zn

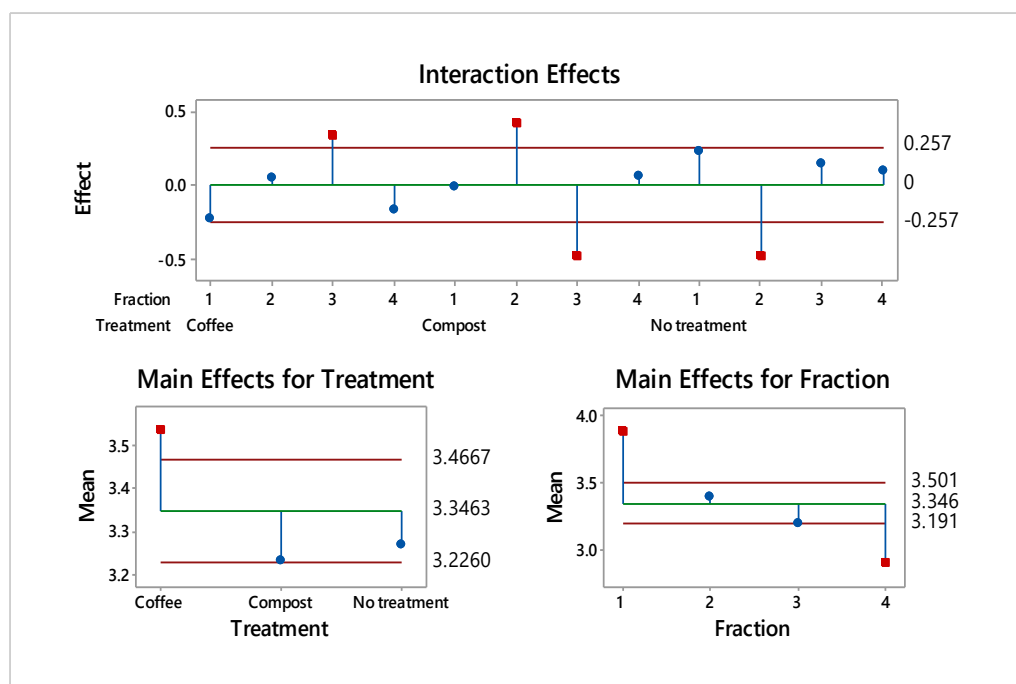


Figure A 8. Two-way normal analysis of means for log transformed Pb BCR fractions applied to the Tyndrum field trial sampled tailings in Chapter 7.

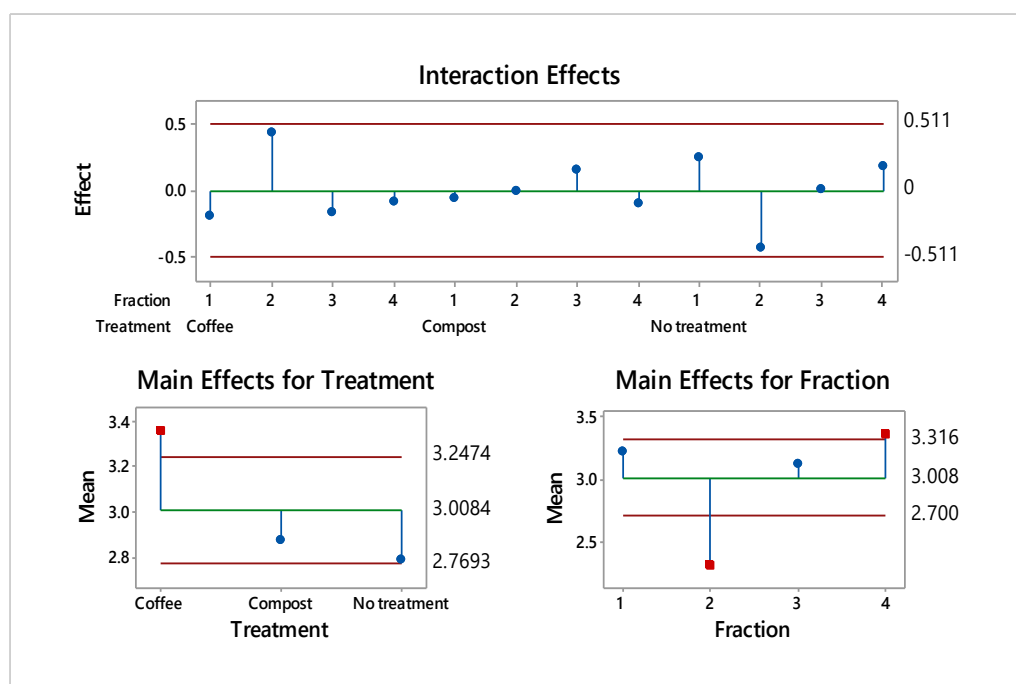


Figure A 9. Two-way normal analysis of means for log transformed Zn BCR fractions applied to the Tyndrum field trial sampled tailings in Chapter 7.

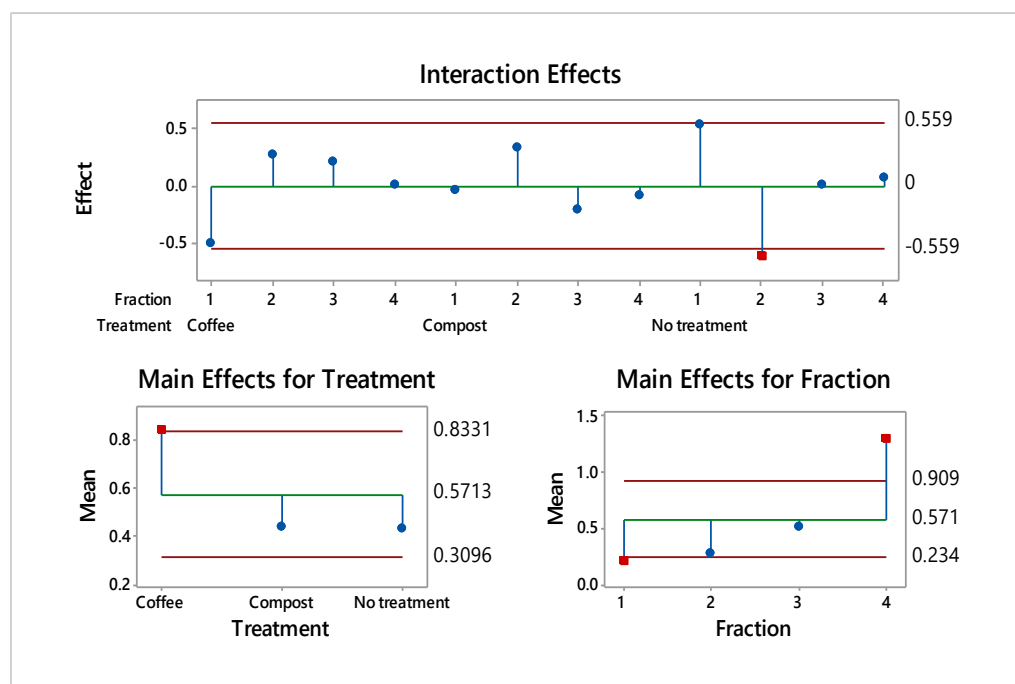


Figure A 10. Two-way normal analysis of means for log transformed Cd BCR fractions applied to the Tyndrum field trial sampled tailings in Chapter 7.

Appendix 7. Correlation Matrix of PTE Measured Parameters (Chapter 7)

Tyndrum

Table A 22. Pearson's product-moment correlation coefficient for Pb measured parameters and tailing characteristics at the Tyndrum tailings. WS-P = Water soluble phosphorous, PS = Particle size. Correlation co-efficients greater than 0.7 or less than -0.7 are high

	BCR-1 Pb	CaCl ₂ Pb	Total Pb	Shoot Pb	Root Pb	Plant total Pb	TN	TC	pH	CWE C	HWE C	NO ₃ -	NH ₄ +	PS	WS- P
BCR-1 Pb	1.00														
CaCl ₂ Pb	0.30	1.00													
Total Pb	0.86	0.26	1.00												
Pb Shoot	0.79	0.19	0.47	1.00											
Pb Root	0.97	0.33	0.89	0.67	1.00										
Plant total Pb	0.99	0.32	0.85	0.77	0.99	1.00									
TN	-0.24	-0.02	0.08	-0.57	0.12	-0.22	1.00								
TC	-0.10	0.15	0.23	-0.47	0.01	-0.09	0.79	1.00							
pH	-0.26	-0.76	-0.28	-0.06	0.32	-0.29	0.16	0.50	1.00						
CWEC	-0.02	-0.18	0.09	-0.33	0.10	0.02	0.05	0.23	0.04	1.00					
HWEC	-0.14	-0.08	0.03	-0.48	0.00	-0.09	0.36	0.55	0.27	0.83	1.00				
NO ₃ ⁻	-0.08	-0.01	0.18	-0.30	0.05	-0.10	0.81	0.51	0.06	-0.42	-0.13	1.00			
NH ₄ ⁺	-0.57	-0.34	-0.18	-0.56	0.52	-0.55	0.79	0.54	0.35	-0.46	-0.12	0.84	1.00		
PS	-0.52	-0.59	-0.44	-0.55	0.52	-0.56	0.05	0.20	0.56	0.18	-0.04	0.03	0.06	1.0 0 0.2	
WS-P	-0.22	-0.34	-0.10	-0.29	0.23	-0.25	0.35	0.11	0.15	-0.18	0.02	0.46	0.44	0	1.00

Table A 23. Pearson's product-moment correlation coefficient for Zn measured parameters and tailing characteristics at the Tyndrum tailings. WS-P = Water soluble phosphorous, PS = Particle size. Correlation co-efficients greater than 0.7 or less than -0.7 are high

	BCR-1 Zn	CaCl ₂ Zn	Total Zn	Shoot Zn	Root Zn	Plant total Zn	TN	TC	pH	CWE C	HWE C	NO ₃ -	NH ₄ +	PS	WS- P
BCR-1 Zn	1.00														
CaCl ₂ Zn	0.37	1.00													
Total Zn	0.92	0.32	1.00												

Zn Shoot	0.85	0.48	0.87	1.00												
Zn Root	0.94	0.42	0.98	0.94	1.00											
Plant total Zn	0.92	0.45	0.95	0.98	0.99	1.00										
TN	-0.21	-0.20	0.05	-0.07	0.06	-0.07	1.00									
TC	-0.26	-0.03	0.12	-0.04	0.13	-0.09	0.79	1.00								
pH	0.30	-0.06	0.33	0.20	0.27	0.25	0.16	0.50	1.00							
CWEC	0.03	0.35	0.07	0.29	0.15	0.21	0.05	0.23	0.04	1.00						
HWEC	-0.23	0.19	0.17	-0.01	0.12	-0.08	0.36	0.55	0.27	0.83	1.00					
NO ₃ ⁻	-0.14	-0.15	0.05	-0.10	0.02	-0.05	0.81	0.51	0.06	-0.42	-0.13	1.00				
NH ₄ ⁺	-0.18	-0.69	0.04	-0.27	0.19	-0.22	0.79	0.54	0.35	-0.46	-0.12	0.84	1.00			
PS	0.34	0.07	0.50	0.29	0.45	0.40	0.05	0.20	0.56	0.18	-0.04	0.03	0.06	1.0	0	
WS-P	-0.01	-0.45	0.24	-0.10	0.09	0.02	0.35	0.11	0.15	-0.18	0.02	0.46	0.44	0	1.00	

Table A 24. Pearson's product-moment correlation coefficient for Cd measured parameters and tailing characteristics at the Tyndrum tailings. WS-P = Water soluble phosphorous, PS = Particle size. Correlation co-efficients greater than 0.7 or less than -0.7 are high

	BCR-1 Cd	CaCl₂ Cd	Total Cd	Shoot Cd	Root Cd	Plant total Cd	TN	TC	pH	CWE C	HWE C	NO ₃ -	NH ₄ +	PS	WS- P
BCR-1 Cd	1.00														
CaCl ₂ Cd	0.07	1.00													
Total Cd	0.64	0.28	1.00												
Cd Shoot	0.41	0.05	0.14	1.00											
Cd Root	0.28	0.09	0.17	0.91	1.00										
Plant total Cd	0.33	0.08	0.17	0.96	0.99	1.00									
TN	-0.41	-0.16	0.05	-0.43	0.37	-0.40	1.00								
TC	-0.40	0.02	0.06	-0.36	0.20	-0.25	0.79	1.00							
pH	0.22	0.22	0.23	0.07	0.01	0.03	0.16	0.50	1.00						
CWEC	-0.03	0.17	0.08	-0.22	0.23	-0.23	0.05	0.23	0.04	1.00					
HWEC	-0.23	-0.01	0.15	-0.33	0.24	-0.27	0.36	0.55	0.27	0.83	1.00				
NO ₃ ⁻	-0.34	-0.07	0.04	-0.45	0.32	-0.37	0.81	0.51	0.06	-0.42	-0.13	1.00			
NH ₄ ⁺	-0.50	-0.22	0.08	-0.09	0.11	0.07	0.79	0.54	0.35	-0.46	-0.12	0.84	1.00		
PS	0.14	-0.25	0.35	-0.19	0.23	-0.22	0.05	0.20	0.56	0.18	-0.04	0.03	0.06	1.0	0
WS-P	-0.20	-0.34	0.19	-0.11	0.13	0.05	0.35	0.11	0.15	-0.18	0.02	0.46	0.44	0	1.00

Le Bleymard

Table A 25. Pearson's product-moment correlation coefficient for Pb measured parameters and tailing characteristics at the Le Bleymard tailings. WS-P = Water soluble phosphorous. Correlation co-efficients greater than 0.7 or less than -0.7 are highlighted in bold

	Total Pb	EDTA A Pb	CaCl ₂ Pb	Shoot Pb	Pb Root Pb	Total Plant Pb	CWE C	HWE C	TC	NO ₃ ⁻	NH ₄ ⁺	TN	pH	WS-P
Total Pb	1.00													
EDTA Pb	0.84	1.00												
CaCl ₂ Pb	0.06	0.04	1.00											
Pb Shoot	0.73	0.68	0.17	1.00										
Pb Root	0.86	0.90	0.13	0.87	1.00									
Total Plant Pb	0.85	0.86	0.14	0.93	0.99	1.00								
CWEC	0.00	0.01	-0.04	0.03	-0.07	-0.04	1.00							
HWEC	-0.10	-0.22	-0.21	-0.39	-0.37	-0.38	0.72	1.00						
TC	-0.11	-0.19	-0.59	-0.04	-0.07	-0.06	0.39	0.23	1.00					
NO ₃ ⁻	0.09	0.09	-0.16	-0.26	0.03	-0.06	-0.11	0.17	0.1	1.00				
NH ₄ ⁺	0.26	0.03	-0.09	0.46	0.18	0.27	0.45	0.12	0.3	0.02	1.00			
TN	-0.11	-0.13	-0.55	-0.15	-0.22	-0.20	0.67	0.71	0.4	-	-	1.00		
pH	0.43	0.09	0.04	0.40	0.35	0.37	-0.33	-0.31	0.8	0.15	0.17	-	1.00	
WS-P	0.02	-0.21	-0.26	-0.31	-0.17	-0.21	-0.10	0.01	0.0	0.15	0.15	0.30	0	
									0.3	-	-	0.23	0.2	
									6	0.13	0.10	0.23	6	1.00

Table A 26. Pearson’s product-moment correlation coefficient for Cd measured parameters and tailing characteristics at the Le Bleymard tailings. WS-P = Water soluble phosphorous. Correlation co-efficients greater than 0.7 or less than -0.7 are highlighted in bold.

	Total Cd	EDT A Cd	CaCl ₂ Cd	Shoot Cd	Root Cd	Total Plant Cd	CWE C	HWE C	TC	NO ₃ ⁻	NH ₄ ⁺	TN	pH	WS-P
Total Cd	1.00													
EDTA Cd	0.47	1.00												
CaCl ₂ Cd	-0.17	-0.21	1.00											
Cd Shoot	0.81	0.39	-0.45	1.00										
Cd Root	0.90	0.33	-0.31	0.90	1.00									
Total Plant Cd	0.88	0.37	-0.37	0.97	0.98	1.00								
CWEC	-0.08	0.03	0.75	-0.31	0.21	-0.26	1.00							
HWEC	-0.43	-0.42	0.81	-0.62	0.46	-0.54	0.72	1.00						
TC	-0.45	0.09	0.40	-0.39	0.42	-0.42	0.39	0.23	1.00					
NO ₃ ⁻	-0.31	-0.79	0.10	-0.28	0.22	-0.25	-0.11	0.17	0.1	1.00				
NH ₄ ⁺	-0.12	0.25	0.25	-0.34	0.34	-0.35	0.45	0.12	0.3	0.02	1.00			
TN	-0.25	0.13	0.77	-0.47	0.31	-0.39	0.67	0.71	0.4	0.15	0.17	1.00		
pH	-0.28	0.05	-0.26	-0.10	0.21	-0.17	-0.33	-0.31	0.0	-	-	-	1.00	
WS-P	-0.24	-0.08	0.08	-0.42	0.30	-0.36	-0.10	0.01	0.3	0.15	0.15	0.30	0.2	1.00
									6	0.13	0.10	0.23	6	

Table A 27. Pearson’s product-moment correlation coefficient for Zn measured parameters and tailing characteristics at the Le Bleymard tailings. WS-P = Water soluble phosphorous. Correlation co-efficients greater than 0.7 or less than -0.7 are highlighted in bold.

	Total Zn	EDT A Zn	CaCl ₂ Zn	Zn Shoot	Zn Root	Total Plant Zn	CWE C	HWE C	TC	NO ₃ ⁻	NH ₄ ⁺	TN	pH	WS-P
Total Zn	1.00													
EDTA Zn	0.63	1.00												
CaCl ₂ Zn	-0.25	-0.31	1.00											
Zn Shoot	0.48	0.42	-0.46	1.00										
Zn Root	0.61	0.60	-0.31	0.72	1.00									
Total Plant Zn	0.60	0.57	-0.39	0.89	0.96	1.00								
CWEC	-0.17	-0.16	0.84	-0.13	-	-0.15	1.00							
HWEC	-0.43	-0.50	0.82	-0.39	-	-0.49	0.72	1.00						
TC	-0.56	-0.36	0.46	-0.58	0.60	-0.64	0.39	0.23	1.00					
NO ₃ ⁻	-0.19	-0.53	-0.06	-0.41	-	-0.50	-0.11	0.17	0.1	1.00				
NH ₄ ⁺	-0.21	-0.02	0.22	-0.05	-	-0.04	0.45	0.12	0.3	0.02	1.00			
TN	-0.38	-0.33	0.86	-0.44	-	-0.45	0.67	0.71	0.4	-	0.17	1.00		
					0.41				8	0.15				

pH	-0.34	-0.29	-0.34	-0.01	0.06	0.03	-0.33	-0.31	0.09	-	0.15	-	1.00	
WS-P	-0.24	-0.37	0.08	-0.47	-	-0.60	-0.10	0.01	0.36	0.13	-0.10	0.23	0.26	1.00

Appendix 9. Plot Variations Influencing Vegetative Biomass at the Tyndrum Mine

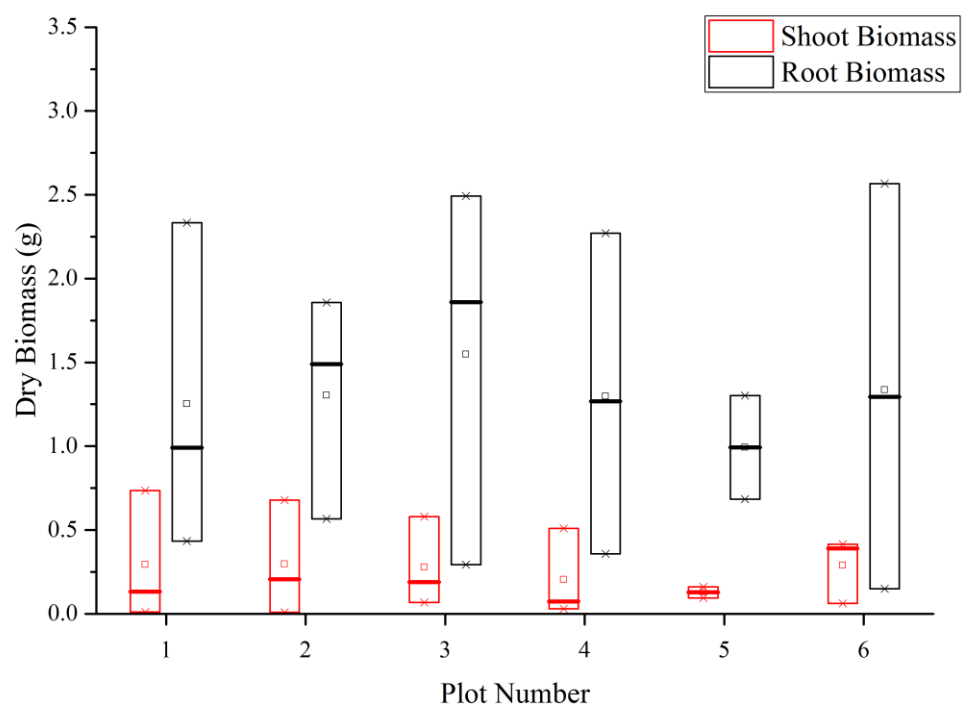


Figure A 11. Root and shoot biomasses of collected vegetation by plot at the Tyndrum mine tailing. Plot 5 appears to have a supressing effect on the range of biomass development across all treatments. No plant growth in the un-treated group was recorded for plot 5.

Appendix 10. Rainfall over the Field Trial Period.

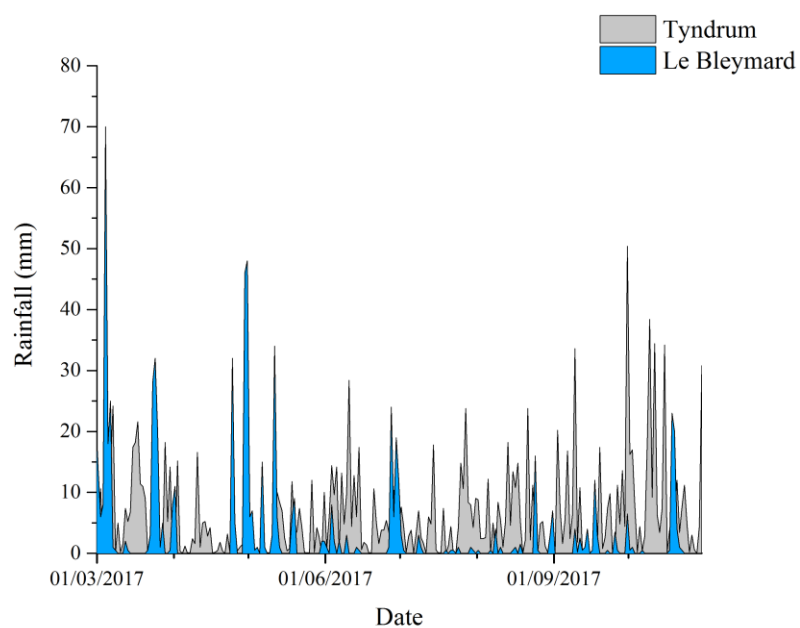


Figure A 12. Recorded precipitation rates approximately 0.5 km south of the Tyndrum and Le Bleymard Pb/Zn mines. Data for Tyndrum collected courtesy of the Met Office from the automatic weather station at Kirkton (Tyndrum No. 3) (grid ref. NN 35949 28385; 170 m.

Appendix 11. PTE Variation across Treatment and Plot

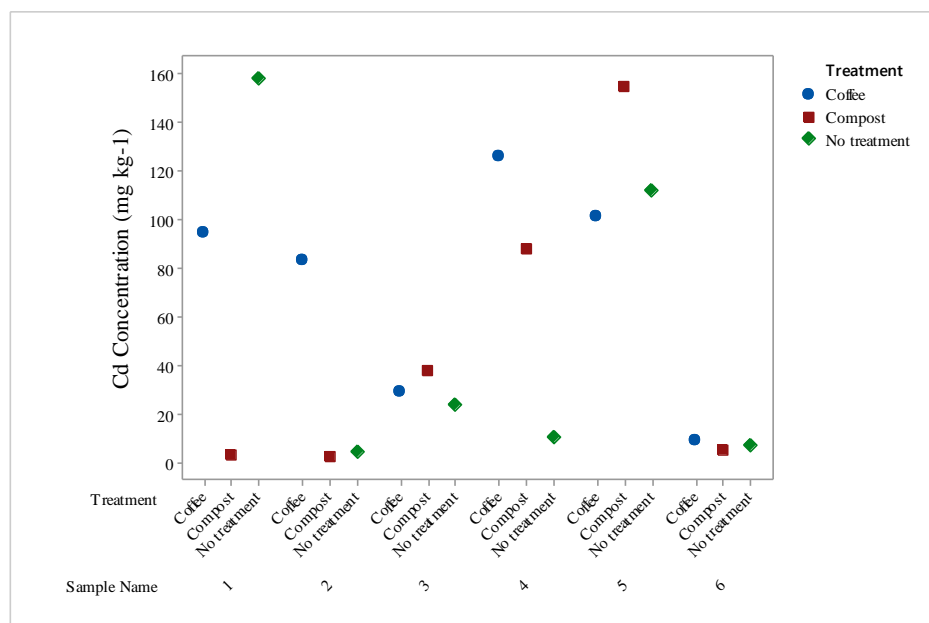


Figure A 13. Total concentrations of Cd by treatment and plot location at the Tyndrum site.

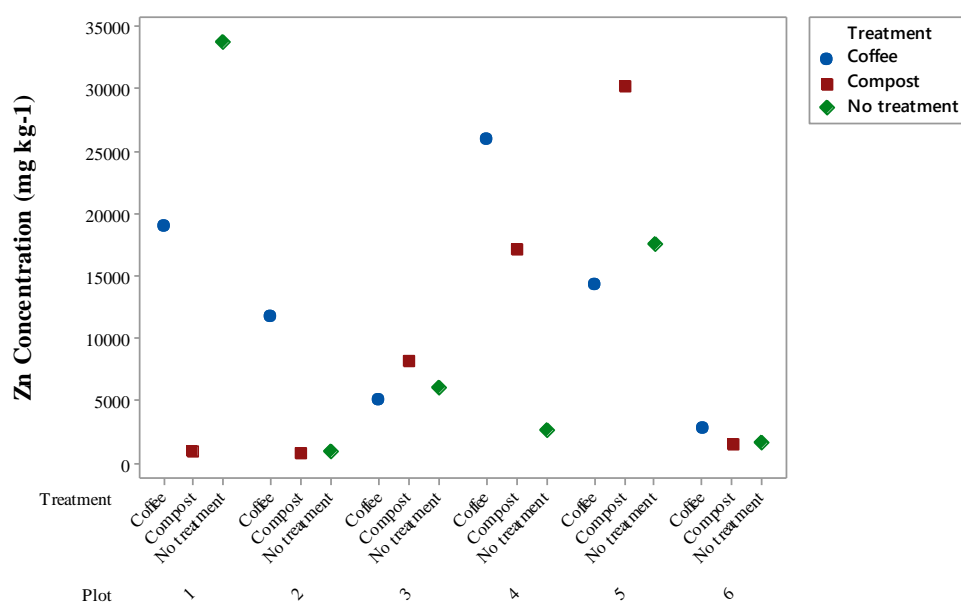


Figure A 14. Total concentrations of Zn by treatment and plot location at the Tyndrum site.

Appendix 12. Total and CaCl₂ – Extractable PTE Concentrations in Spiked Synthesised Mine Tailings and GWC.

Table 8-1. Calcium Chloride extractable Pb and Zn in Phase 1 Tyndrum synthesised mine tailings. n = 5

Treatment Group	CaCl₂ Extractable Pb (mg kg⁻¹)	CaCl₂ Extractable Zn (mg kg⁻¹)	Total Pb (mg kg⁻¹)	Total Zn (mg kg⁻¹)
<i>Agrostis capillaris</i>	217 (± 18)	153 (± 14)	314 (± 10)	386 (± 86)
<i>Agrostis vinealis</i>	197 (± 16)	151 (± 13)	302 (± 12)	347 (± 63)
<i>Capsella bursa-pastoris</i>	222 (± 12)	157 (± 25)	295 (± 15)	378 (± 46)
<i>Festuca Ovina</i>	206 (± 15)	159 (± 29)	305 (± 13)	378 (± 36)
<i>Cardamine hirsuta</i>	216 (± 20)	151 (± 22)	305 (± 6)	368 (± 45)
<i>Molinia caerulea</i>	202 (± 20)	147 (± 14)	319 (± 19)	435 (± 10)
Control	1.8 (± 1.3)	16.9 (± 3.1)	23.5 (± 2.3)	231 (± 8.1)

Table 8-2. Calcium Chloride extractable Pb and Zn in Phase 1 Le Bleymard synthesised mine tailings. n = 5

Treatment Group	CaCl₂ Extractable Pb (mg kg⁻¹)	CaCl₂ Extractable Zn (mg kg⁻¹)	Total Pb (mg kg⁻¹)	Total Zn (mg kg⁻¹)
<i>Agrostis capillaris</i>	< 0.01	21.3 (± 6.4)	25.0 (± 2.7)	290 (± 50)
<i>Agrostis vinealis</i>	< 0.01	21.3 (± 5.0)	23.5 (± 2.4)	247 (± 55)
<i>Capsella bursa-pastoris</i>	< 0.01	18.0 (± 4.4)	24.2 (± 2.4)	287 (± 33)
<i>Festuca ovina</i>	< 0.01	23.8 (± 4.4)	26.4 (± 2.3)	275 (± 37)
<i>Lavandula angustifolia</i>	< 0.01	22.0 (± 5.6)	25.5 (± 4.8)	295 (± 34)
<i>Nocca caerulea</i>	< 0.01	26.5 (± 8.3)	25.6 (± 5.3)	269 (± 21)
Control	< 0.01	16.9 (± 3.1)	25.3 (± 2.7)	207 (± 22)

Table 8-3. Calcium Chloride extractable Pb and Zn in Phase 2 spiked green waste compost. n = 7

Treatment Group	CaCl₂ Extractable Pb (mg kg⁻¹)	CaCl₂ Extractable Zn (mg kg⁻¹)	Total Pb (mg kg⁻¹)	Total Zn (mg kg⁻¹)
<i>A.Capillaris</i>	778 (± 28)	809 (± 39)	870 (± 8.5)	1,060 (± 50)
<i>F.ovina</i>	794 (± 27)	842 (± 28)	865 (± 9.5)	1,000 (± 26)
Mixed	799 (± 34)	830 (± 20)	863 (± 8.7)	1040 (± 30)
Control	0.62 (0.07)	43.5 (16.7)	68.9 (± 1.1)	239 (± 3.8)